U.PORTO

CICLO DE ESTUDOS: Doutoramento

ÁREA DE ESPECIALIZAÇÃO: Nutrição Clínica

Título: Estado nutricional de doentes com mucopolissacaridose

Nutritional status of patients with mucopolysaccharidosis

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2017

Nutritional status of patients with mucopolysaccharidosis

Estado nutricional de doentes com mucopolissacaridose

Academic Dissertation

To be presented to the *Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto*, for public examination for the degree of

PhD in Clinical Nutrition

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Porto

2017

This investigation was supported by:

BioMarin Europe Ltd

Shire

"We are guilty of many errors and many faults, but our worst crime is abandoning the children, neglecting the fountain of life. Many of the things we need can wait. The child cannot. Right now is the time his bones are being formed, his blood is being made, and his senses are being developed. To him we cannot answer 'Tomorrow', his name is today."

Gabriela Mistral, 1948

Aos meus filhos, Carolina, Diogo e Maria.

ACKNOWLEDGEMENTS

À Professora Doutora Luísa Diogo, pelos ensinamentos e apoio constante e por me incentivar a nunca desistir.

À Doutora Paula Garcia, por tudo o que me transmitiu, conhecimentos e amizade.

À Doutora Maria do Carmo Macário, à Professora Doutora Esmeralda Martins, ao Professor Doutor Júlio Rocha, à Doutora Manuela Almeida, à Doutora Elisa Leão Teles, á Doutora Esmeralda Rodrigues, à Doutora Carla Vasconcelos, à Doutora Ana Gaspar e à Doutora Patrícia Janeiro, pela disponibilidade e colaboração na concretização do projeto.

À BioMarin Ltd. (nas pessoas do Doutor Tiago Barros e da Professora Doutora Sónia Albuquerque) e à Shire pelo apoio financeiro, fundamental para a realização da investigação.

À Professora Doutora Florbela Vitória, por toda a ajuda e celeridade nas respostas.

À Ana Serpa Oliveira, pela ajuda na revisão e por toda a disponibilidade que sempre demonstrou.

À Maria Armanda por toda a colaboração cientifica, e pelo apoio neste ultimo ano.

Aos meus verdadeiros Amigos, que estão sempre lá quando grito "socorro!".

Aos meus Pais, por estarem sempre e incondicionalmente disponíveis, abdicando de tudo para tudo nos proporcionar.

Ao Rui, por estar ao meu lado e dos nossos filhos, apesar de todas as horas que não estive por perto.

Aos meus filhos, Carolina, Diogo e Maria, pelos momentos em que precisavam de mim, mas em que não pude estar presente.

Muito Obrigada!

ABSTRACT

Background:

Mucopolysaccharidosis (MPS) are a group of rare, genetic, multisystem, progressive diseases. They are characterized by lysosomal accumulation of glycosaminoglycans, due to congenital enzymatic deficiencies. Patients are susceptible to nutritional status deterioration as a consequence of poor quality of health (inherent to the degenerative character of the disorders), and/or inadequate food habits. Treatment, which is far from optimal, has been focused in symptoms alleviation and search for specific ("curative") therapies. Nutritional issues have not been a major concern in the majority of the studies.

Aim:

The main objective of this study was to evaluate nutritional status of MPS patients, in relation to their food and nutritional intake and quality of health.

Methods:

This is a cross-sectional study that included patients from four Portuguese Lysosomal Disorders Treatment Centers. Demographic, recent (24-hour recall) and usual (food-frequency questionnaire) food intake data were collected. Anthropometric results (weight, height, body mass index) were compared with WHO references. Body composition was assessed by tetrapolar biompedance analysis. Plasma proteins, vitamins, minerals, lipids levels and essential fatty acids, as well as renal and liver function tests, total blood count and phosphocalcium metabolism were evaluated. The Health Assessment Questionnaire (HAQ) was applied to patients (or caregivers). The 6-minute walking test (6-MWT) was performed by a subgroup of patients.

This study was approved by the Ethical Committees of each Treatment Centre, and *Comissão Nacional de Proteção de Dados*. Patients, parents or other legal guardians gave a written informed consent.

Results:

Thirty-one patients (five MPS I; four MPS II; nine MPS III; three MPS IV; nine MPS VI; one MPS VII), aged between 1.7 and 32.7 years, were included. Mean age at diagnosis was 3.2 (SD=3.0) years. Nineteen patients (nine MPS VI) were under enzyme replacement therapy (ERT). Ten were taking nutritional supplements, mainly vitamin D formulas. A high number of patients drank sugar-rich beverages on a weekly basis and/or did not eat fruits and vegetables regularly.

Caloric, protein, carbohydrates and fiber intakes were lower than estimated average requirements (EAR) in 56.0%, 32.0%, 64.0% and 76.0% of the subjects,

respectively. Fat intake was adequate in 52.0% and high in 36.0%. The intake of vitamins and minerals was high in most patients.

Weight z-score varied from -1.7 to 2.6, height z-score, from -10.1 to 1.5 and BMI z-score, from -4.6 to 3.4. The lowest values were observed in MPS VI, MPS IV and MPS I groups for weight, height and BMI, respectively. Two patients presented a status of severe thinness and six showed overweight, (one obese). Minimum mean body fat mass (16.6%) and body lean mass (13.8%) were displayed in MPS VI and in MPS IV groups, respectively. Phase-angle varied from 3.0 to 6.2°.

Pre-albumin, retinol binding protein (RBP), creatinine and HDL-cholesterol were low in 59.3%, 75.0%, 77.4% and 48.4%, of the patients, respectively. Vitamin D insufficiency was presented in 38.7% and deficiency in 48.4%. MPS III group showed significantly higher plasma pre-albumin RBP and vitamin A levels, than the other groups. MPS VI group exhibited lower RBP, vitamin A and vitamin E than the others. MPS IV group presented higher vitamin E levels than the remainder. Plasma pre-albumin and vitamin A were significantly lower in patients submitted to ERT, compared to others.

Mean total HAQ score (part I) was 6.6/11 (SD=3.8) (11=total inability). Activities related to dressing and bathing were associated with the greatest difficulties. Moderate positive correlations were detected between age and scores of: "eating and drinking" "toileting", "mobility", and "walking and climbing stairs". A moderate positive and significant correlation was found between age and total HAQ score. Strong negative correlations were displayed between "walking and climbing stairs" scores and plasma creatinine and araquidonic acid levels. MPS type III patients had higher scores in "eating and drinking", "dressing" and total HAQ than the other MPS groups. Caregiver assistance total score (HAQ - part II) presented a mean of 3.0/11 (SD=1.1). MPS type II patients showed significantly higher results in 6-MWT than the other groups.

Discussion and conclusion

Healthy food (fruits and vegetables) is not regularly consumed by most of the patients, whereas sugar-rich drinks are often present in the patients' diet. The majority had lower caloric, carbohydrates and fiber intake and a great part showed lower protein and higher fat intake than recommendations.

A great number of patients presented nutritional status indicators in levels lower than normal: phase-angle, pre-albumin, RBP and creatinine. Low levels of plasma vitamin D were most prevalent.

MPS VI patients showed lower RBP, vitamin A and vitamin E. The former two were also lower in the group submitted to ERT (where about half of the patients were MPS VI).

A high level of difficulties was found in the sample, associated with high dependence scores. Older patients showed more difficulties, in general. MPS III patients were the ones were difficulties were the greatest in global test, namely

in “eating and drinking” and “dressing” domains. Higher levels of dependence were also present in patients with lower plasma creatinine and araquidonic acid levels.

A high level of disease severity was found in this group of patients. In a substantial part of them, nutritional status indicators were found to be abnormal, pointing to a hypothetical malnutrition status, which could not be proved by our results. A concurrent factor for the abnormalities found in nutritional status indicators could be the latent status of chronic inflammation, known to be associated to these diseases. Unfortunately, inflammation status of the patients was not included in the aims of the present investigation. More studies, with larger samples and including other parameters are required, in order to better clarify our results.

A protocol of nutritional surveillance for the adequate follow-up of MPS is suggested. The multidisciplinary team in charge of these patients should include a specialized nutritionist.

Keywords: mucopolysaccharidosis; nutrition; body composition; quality of health.

RESUMO

Introdução

As mucopolissacaridoses (MPS) são um grupo de doenças genéticas, raras, progressivas com envolvimento multisistémico. Caraterizam-se por uma acumulação lisosomal de glicosaminoglicanos, causada por défices enzimáticos específicos. A debilidade do estado de saúde (inerente à natureza degenerativa destas doenças) e/ou hábitos alimentares possivelmente desadequados, conferem a estes doentes grande suscetibilidade de deterioração do seu estado nutricional. O tratamento, longe de ser o mais adequado, tem-se focado essencialmente nas co-morbilidades, enquanto se investe ativamente no desenvolvimento e aperfeiçoamento de terapêuticas especificas, "curativas". O estado nutricional destes doentes não tem, de um modo geral, sido objeto de investigação.

Objetivos:

O principal objetivo deste estudo foi avaliar o estado nutricional de doentes com MPS, relacionando-o com a ingestão alimentar e nutricional e com o seu estado de saúde.

Métodos:

Trata-se de um estudo transversal para o qual foram convidados a participar doentes de quatro Centros de Tratamento de Doenças do Lisossoma Portugueses. Recolheu-se dados demográficos, assim como informação sobre a ingestão alimentar recente (24 horas anteriores) e habitual (questionário de frequência alimentar). Os dados antropométricos foram avaliados com recurso aos parâmetros de referencia da OMS. A composição corporal foi estimada através de bioimpedância elétrica tetrapolar. Analisou-se igualmente: proteínas, vitaminas, minerais, lípidos e ácidos gordos essenciais plasmáticos, hemograma e alguns parâmetros de função renal e hepática e do metabolismo fosfo-cálcico. O Questionário de Avaliação do Estado de Saúde (HAQ) foi igualmente respondido pelos doentes (ou cuidadores). Um subgrupo dos doentes executou o teste de caminhada de 6 minutos.

Este estudo foi aprovado pelas Comissões de Ética de cada Centro de Tratamento e pela Comissão Nacional de Proteção de Dados. Todos os doentes, pais ou tutores legais deram consentimento informado escrito para a participação.

Resultados:

Foram avaliados trinta e um doentes (cinco MPS I; quatro MPS II; nove MPS III; três MPS IV; nove MPS VI; um MPS VII), com idades compreendidas entre 1,7 e 32,7 anos. O diagnostico foi realizado em média aos 3,2 anos (DP=3,0). Dezanove doentes (nove MPS VI) estavam sob terapia de substituição enzimática (ERT). Dez

referiram a toma de suplementos nutricionais, principalmente de fórmulas de vitamina D. Um significativo numero de doentes consumia bebidas açucaradas semanalmente e / ou não ingeria fruta e legumes regularmente. A ingestão calórica, proteica, glicídica e de fibras era inferior às recomendações ("EAR") em 56,0%, 32,0%, 64,0% e 76,0% dos indivíduos, respetivamente. A ingestão de lípidos era adequada em 52,0% e superior ao recomendado em 36,0%. A maioria dos doentes apresentava uma ingestão de vitaminas e minerais superior às necessidades.

Os indicadores z-score do peso variaram de -1,7 a 2,6, os do, z-score da estatura, entre -10,1 e 1,5 e os do z-score do índice de massa corporal, de -4,6 a 3,4. foram os mais baixos, respetivamente Os valores mais baixos foram observados nos grupos com MPS VI, MPS IV e MPS I, respetivamente para o peso, a estatura e o índice de massa corporal. Dois doentes apresentavam magreza extrema e seis, excesso de peso (um obeso). Os grupos com MPS VI e MPS IV tinham os mínimos valores médios de massa gorda (16,6%) e de massa magra (13,8%), respetivamente. O ângulo de fase variou entre 3,0 e 6,2°. Os níveis de pré-albumina, proteína transportadora do retinol (RBP), creatinina e colesterol-HDL eram baixos em, respetivamente, 59,3%, 75,0%, 77,4% e 48,4% dos doentes. Encontrou-se insuficiência de vitamina D em 38,7% dos indivíduos e deficiência em 48,4%.

Os doentes com MPS III apresentaram valores de pré-albumina, RBP e vitamina A plasmáticos significativamente superiores aos dos restantes grupos. No grupo com MPS VI foram encontrados valores de RBP, vitamina A e vitamina E inferiores aos restantes. Os doentes com MPS IV apresentaram níveis de vitamina E plasmática superiores aos dos restantes grupos. A pré-albumina e a vitamina A plasmáticas eram significativamente inferiores nos doentes submetidos a ERT, relativamente aos restantes.

A pontuação média do questionário HAQ (parte I) foi de 6,6/11 (DP=3,8) (11=incapacidade total). As atividades relacionadas com o vestir e a higiene pessoal estavam associadas a maiores dificuldades. Foram encontradas correlações positivas moderadas entre a idade e as pontuações de: "comer e beber", "higiene pessoal", "mobilidade", "caminhar e subir escadas". Entre a idade e a pontuação média total do HAQ foi identificada uma correlação positiva e moderada, estatisticamente significativa. Foram ainda estabelecidas correlações negativas fortes entre a pontuação do domínio "caminhar e subir escadas" e os valores plasmáticos de creatinina e do ácido araquidónico. Os doentes com MPS III apresentaram pontuações mais elevadas nos domínios "comer e beber" e "vestir" e na pontuação global do HAQ, relativamente aos restantes grupos. A pontuação média relativamente à dependência do cuidador (HAQ – parte II) foi de 3,0/11 (DP=1,1). Os doentes com MPS II mostraram, em relação aos outros grupos, resultados significativamente superiores na prova da caminhada.

Discussão e conclusão

A generalidade dos doentes não consumia regularmente alimentos saudáveis, como frutas e vegetais e ingeria bebidas açucaradas com regularidade. A ingestão energética, glicídica e de fibras era inferior às recomendações na maioria dos

casos. Uma grande parte dos indivíduos tinha uma ingestão de proteínas e de lípidos, respetivamente inferior e superior às necessidades.

Alguns indicadores do estado nutricional encontravam-se baixos em muitos dos doentes analisados, nomeadamente o ângulo de fase e a pré-albumina, a RBP e a creatinina plasmáticas. Igualmente prevalentes, foram os níveis diminuídos de vitamina D no plasma. Os doentes com MPS VI apresentavam valores plasmáticos baixos de RBP, vitamina A e vitamina E. No grupo submetido a ERT (no qual cerca de metade dos doentes era MPS VI), havia igualmente valores dos dois primeiros.

A amostra estudada demonstrou um alto nível de dificuldade: média de 6,6, associado com altos níveis de dependência do cuidador. Os doentes mais velhos manifestaram globalmente maiores dificuldades. Nos doentes com MPS III, estas dificuldades foram superiores às dos restantes, e particularmente nos domínios "comer e beber" e "vestir". Nos indivíduos com baixos valores de creatinina e de ácido araquidónico plasmáticos, foram igualmente encontrados maiores níveis de dependência.

Este grupo de doentes apresenta um elevado nível de gravidade. Numa parte significativa da amostra foram encontradas alterações nos indicadores do estado nutricional, apontando para um possível estado de desnutrição, que não pudemos provar com os resultados obtidos. Concomitantemente, poderá existir um estado de inflamação crónica, reconhecidamente associado a estas doenças. No entanto, a avaliação do estado inflamatório dos doentes não foi incluída nos objetivos da presente investigação.

São necessários mais estudos, com amostras de maior dimensão e inclusão de outros parâmetros, de modo a clarificar os dados obtidos.

Propõe-se um protocolo de vigilância nutricional para o seguimento dos doentes com MPS. A equipa multidisciplinar que acompanha estes doentes deve incluir um nutricionista com formação especifica em doenças hereditárias do metabolismo.

Palavras-chave: *mucopolissacaridoses; nutrição; composição corporal; estado de saúde.*

CONTENTS

ABBREVIATIONS

BMI - body mass index

EAR - estimated average requirements

ERT - enzymatic replacement therapy

HAQ - Health Assessment Questionnaire

GAG - glycosaminoglycan

MPS - mucopolysaccharidosis

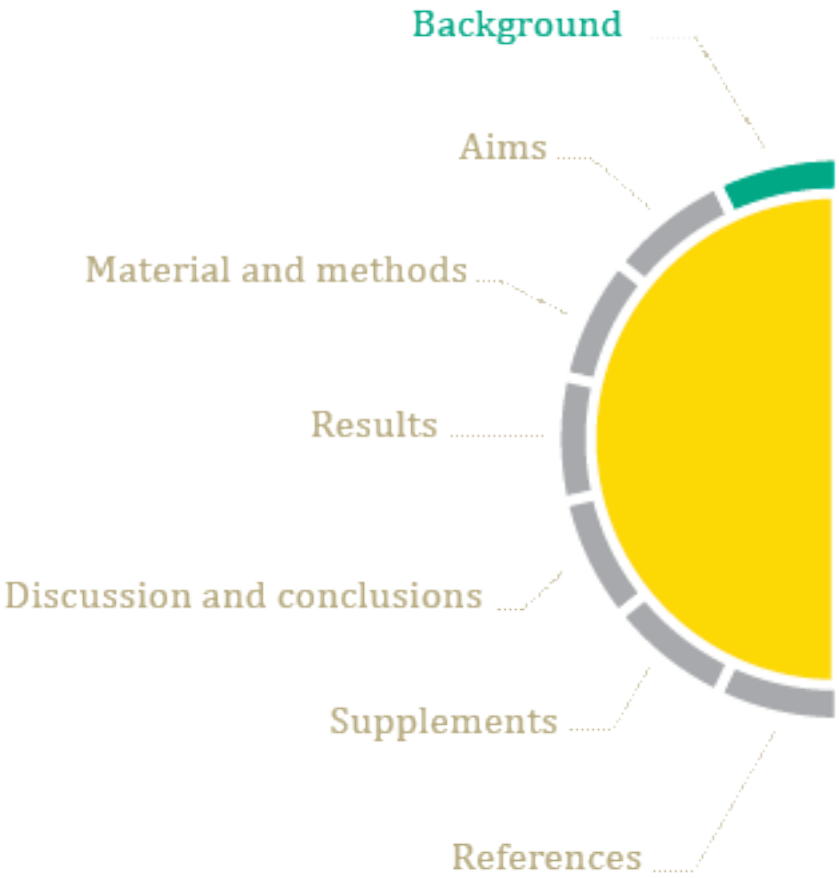
RBP - retinol binding protein

WHO - World Health Organization

6-MWT - six-minute walking test

LIST OF FIGURES

LIST OF TABLES


Background
Aims
Material and methods
Results
Discussion and conclusions
Supplements
References

Mucopolysaccharidosis

Mucopolysaccharidosis (MPS) are a group of genetic, progressive diseases, caused by enzymatic deficiencies on lysosomal degradation of glycosaminoglycans (GAGs) [1].

MPS are orphan diseases, (incidence lower than 1:2 000 live births). Their global incidence ranges from 1.4/10 000 to 1/18 000 [2, 3]. It is estimated to be about 1:25 000 in Portugal [4].

GAGs are part of the structure of the proteoglycans, which are important components of the extracellular matrix (or connective tissue) [5, 6]. They are subject to constant turnover, which is much slower in adults than in children.

Several acid hydrolases act sequentially in lysosomal degradation of GAGs. Deficiency of several of those enzymes are known to cause a block in GAGs catabolic pathways, with progressive accumulation of the respective substrate within cells of various organs. These include liver, spleen, bone, skin and central nervous system. Phenotypes vary according to the specific enzymatic deficiency and its severity, which determine the type, intensity and localization of GAGs storage [5, 7].

Seven major types of MPS are recognized, according to the enzyme defect and the glycosaminoglycans excreted in urine: Hurler-Scheie (MPS I_H and MPS I_S), Hunter (MPS II), Sanfilippo (MPS III; subtypes A, B, C and D), Morquio (MPS IV; subtypes A and B), Maroteaux-Lamy (MPS VI), Sly (MPS VII) and Hyaluronidase deficiency (MPS IX) [5] (Table 1). All MPS types are autosomal recessive disorders, except for MPS II, which is X-linked [8].

Diagnosis of MPS is based on clinical suspicion and confirmed by urine GAGs quantification and electrophoresis analysis, enzymatic assay of lysosomal hydrolases in leucocytes or fibroblasts and genetic molecular study [1, 5] (Table 1).

GAGs accumulation affect predominantly liver and spleen (hepatosplenomegaly), skeletal structure (dysostosis multiplex) and general morphogenesis, with progressive coursing of features, specifically facial. Eye (corneal clouding and glaucoma), brain (psychomotor development delay and/or regression, intellectual deficiency, behavioural disturbances and/or dementia) and heart (cardiomyopathy and valve disease) involvement are also seen, mostly in specific types.

Clinical phenotypes are protean concerning age onset, type and degree of affected organs and disease severity. In general, severity correlates inversely with age at presentation [9].

MPS type	Disease name	Enzymatic defect	Urinary metabolites
MPS I	**Hurler, Scheie, Hurler-Scheie**	α-L-Iduronidase	Dermatan sulfate, heparan sulfate
MPS II	**Hunter**	Iduronate sulfatase	Dermatan sulfate, heparan sulfate
MPS IIIA	**Sanfilippo A**	Heparan sulfate N-sulfatase (sulfamidase)	Heparan sulfate
MPS IIIB	**Sanfilippo B**	α-N-Acetylglucosaminidase	Heparan sulfate
MPS IIIC	**Sanfilippo C**	Acetyltransferase	Heparan sulfate
MPS IIID	**Sanfilippo D**	N-Acetylglucosamine 6-sulfatase	Heparan sulfate
MPS IVA	**Morquio A**	Galactosamine 6-sulfatase	Keratan sulfate, chondroitin 6-sulfate
MPS IVB	**Morquio B**	β-Galactosidase	Keratan sulfate
MPS VI	**Maroteaux-Lamy**	N-Acetylglucosamine 4-sulfatase (arylsulfatase B)	Dermatan sulfate
MPS VII	**Sly**	β-Glucuronidase	Dermatan sulfate, heparan sulfate, chondroitin 4-sulfate, chondroitin 6-sulfate
MPS IX	**-**	Hyaluronidase	Hyaluronic acid

Table 1. Biochemical defects and urinary metabolites for diagnosis of different types of MPS (adapted from Inborn metabolic diseases [1]).

Usually, affected subjects seem normal in early life and develop the typical clinical phenotypes progressively after a few months or years. However, in the most severe forms, they may present typical signs in the neonatal, or even antenatal period. On the other end of the disease spectrum, manifestations emerge in youth or even adult life [10].

Characteristically, MPS I_H, MPS II and MPS VI are more severe, with coarse facies and ENT obstructive disease. MPS VII is frequently associated with *hydrops foetalis* or neonatal massive hepatosplenomegaly and abdominal hernia, although late-onset forms are known [5].

Severe skeletal dysplasia, leading to impairment of growth (stature), and preserved intellectual function, are typically seen in MPS IV and also MPS VI [5]. Cognitive impairment is variable and typical of MPS I, MPS II, all subtypes of MPS III and MPS VII patients, and progressive deterioration can occur. Behavioural disturbances and dementia occur mostly in MPS III (A, B or C) [1].

MPS IX is extremely rare, and patients commonly present with joint involvement [5].

With the improvement of diagnosis techniques, less severely affected patients are being detected, without significant dysmorphism or organomegaly and normal to borderline cognitive levels [11, 12].

In MPS patients, several issues inherent to the consequences of cell storage of GAGs, such as frequent ear infections, sleep apnoea and mechanical compression of the stomach by the

enlarged liver and spleen contribute to nutritional deficiencies and failure to thrive, commonly seen in these subjects. Behavioural disturbances with hyperkinesia and insomnia, depression, cognitive impairment/dementia and heart failure are additional risk factors to malnutrition.

Treatment of MPS patients is mostly supportive, regardless the type. Hematopoietic stem cell transplantation is an available possibility. It has been used with some success in MPS I_H patients, less than 2 years of age. Enzymatic replacement therapy (ERT) is accessible for patients with MPS $I_{H/S}$, MPS II, MPS IVA and MPS VI, and in progress for MPS VII and MPS IIIB [5, 6, 13-15]. Although some clinical improvement has been achieved with both therapeutic methods, they do not halt or revert all aspects of disease progression. In fact, none of the available therapeutic options is disease curative, but, at their best, disease modifiers.

Independently from the MPS type and available specific treatment, all patients need symptomatic treatment and support conducted by multidisciplinary teams, in order to promote well-being and improve quality of life [16-20]. Adequate nutrition is one of the pillars of the therapeutic strategy.

Nutritional status and quality of life in chronic diseases

Growth and nutritional status are excellent indicators of health in children and adolescents.

When a chronic disease like MPS is present, both growth and nutritional status may be impaired due to changes in metabolism secondary to disease, associated with other factors like inability to suck, chew, swallow and digest food properly, oesophageal reflux or the effects of prolonged use of medications [21-23].

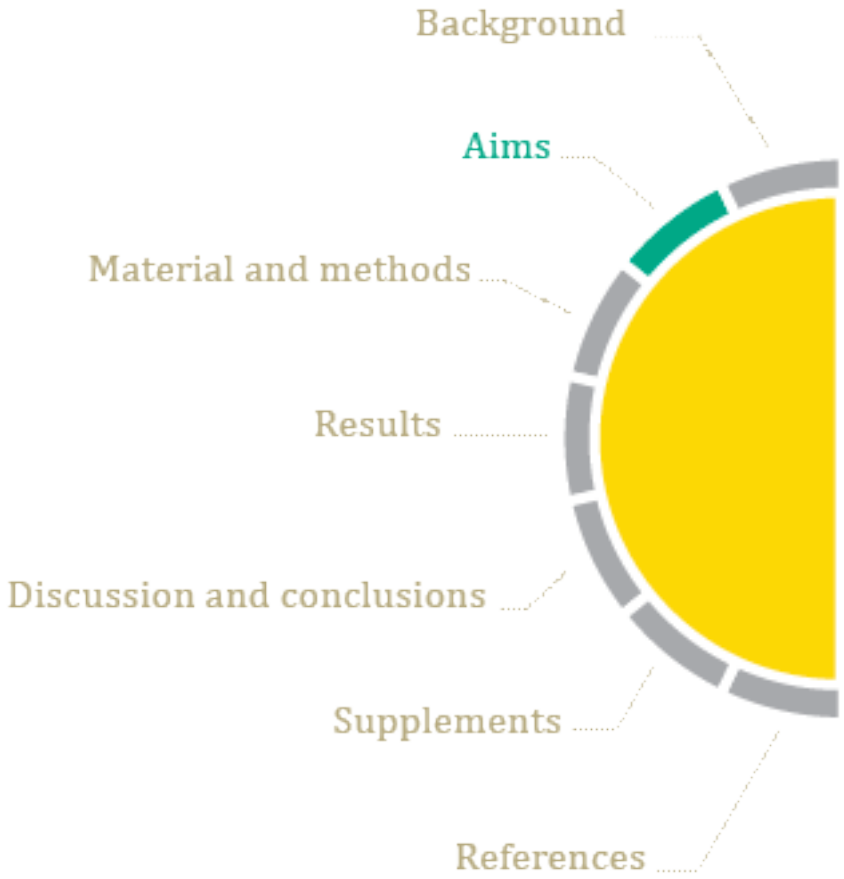
Chronic symptoms (like pain, insomnia, abdominal distension, intellectual disability, feeding or mobility difficulties, to mention a few) and their negative repercussion in nutritional status may have consequences on the quality of life of patients and their families. Providing good nutritional monitoring and support, as well as careful symptomatic treatment, along with anticipation of complications, can contribute to improve patients and families' quality of life.

Assessment of nutritional status

Assessment of nutritional status may lead to identification of the impact of the disease on nourishment, enabling a prompt intervention if needed. It can be achieved through anthropometrics, body composition and laboratorial parameters evaluation. Additionally,

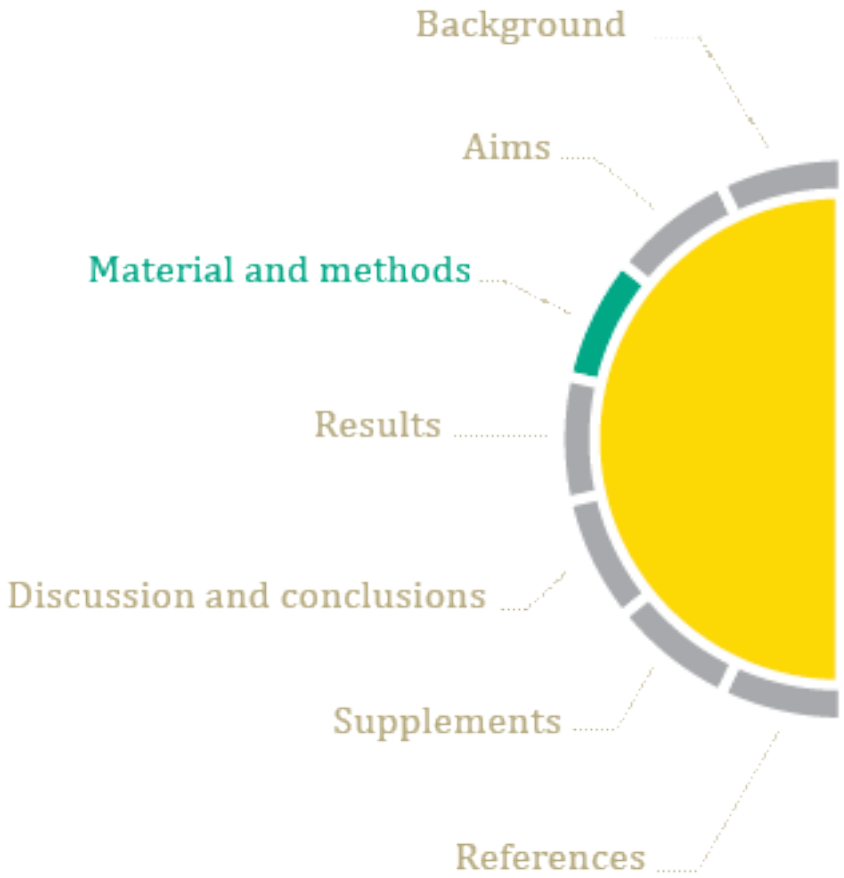
information on usual food and nutritional intake may also help to identify the possible role of unhealthy food choices enhancing disease related factors in nutritional deficiency.

Growth impairment (short stature) due to skeletal dysplasia present in many patients is one of the main debated issues on MPS. However, their nutritional status is rarely discussed, literature on that subject being scarce. This occurs probably because these patients have so many other life threatening complications that nutrition comes second. On the other hand, the "puffy" appearance of most MPS patients due to skin storage may mask nutritional problems, at some extent.


Background
Aims
Material and methods
Results
Discussion and conclusions
Supplements
References

This investigation aimed to:

- Assess nutritional status of patients with MPS of different types;
- Characterize food habits and usual nutritional intake in these individuals;
- Evaluate their health status in association with disease type and/or progression;
- Clarify the impact of disease in dietary intake and nutritional status;
- Establish the link between nutritional intake and nutritional status in patients;
- Correlate autonomy with dietary intake and nutritional status;
- Propose a nutritional protocol to care MPS patients in daily clinics.


Background
Aims
Material and methods
Results
Discussion and conclusions
Supplements
References

PATIENTS

MPS patients from four Lysosomal Disorders Treatment Centers in Portugal were invited to participate in the present study: *Centro Hospitalar Universitário de Coimbra, E.P.E.*, *Centro Hospitalar de São João* - Porto, *Centro Hospitalar do Porto, E.P.E.* and *Centro Hospitalar de Lisboa Norte, E.P.E.*.

A preliminary contact was made between the investigator and the medical teams of the Lysosomal Disorders Treatment Centers collaborating in the study.

Demographic data, MPS type and age at diagnosis were collected.

A code composed by MPS type and a digit (i.e. MPS III_1) was attributed to each patient (Table S1).

METHODS

I. FOOD AND NUTRITIONAL INTAKE

The investigator applied a 24-hour recall questionnaire, adapted to parents or patients, when possible. Information was collected using the available Manual of Quantification of Portuguese Foods (FCNAUP) [24]. Data analysis, detailed by different nutrients, was done according to Portuguese Food Composition Table (INSA) [25].

After accomplishing the previous assessments, in order to assess usual dietary intake over the previous 12 months, a food frequency questionnaire was delivered and its fulfilment was asked for, as recommended by the authors [26-28]. Specific guidelines were explained. No intervention of the investigator occurred during the time patients or parents answered the questionnaires.

Eighty-two food items were included in the questionnaire. Others were included, if consumed in a frequency higher than once per week. Nine possibilities of frequency of intake were considered, from "never or less than once per month" to "six or more times per day", considering the medium serving size presented next to the item. When analysing data, foods with mentioned intake as "never or less than once per month" were excluded. Food intake was calculated multiplying frequencies by standard portion size of the food item. An extra seasonal factor of 0.25 was used, considering seasonality as corresponding to 3 months per year [26, 27].

Food intake conversion into nutrients was made using an adapted Portuguese version of software Food Processor Plus (ESHA Research, USA) and Portuguese Food Composition Table (INSA) [25, 29].

Nutritional intake was compared with Estimated Average Requirement (EAR), or adequate intake (AI) if EAR was inexistent [30-35]. Intakes were considered adequate when found between 90 to 110% of EAR / AI, inclusive. Ingestion lower than 90% of EAR /AI and higher than 100% were considered lower and higher than recommendations, respectively [36].

II. ANTHROPOMETRICS AND BODY COMPOSITION

In each Hospital, patient's weight and height were obtained by standard techniques in the same day of the remaining protocol procedures [37]. For weight determination, different scales were used, according to local availability and the patient's capacity to stand on vertical position. Equipment adapted to bed was used when ability to stand up was impaired. Weight was recorded in kilograms (Kg), adjusted to one decimal place.

Height was measured by standard techniques with the stadiometers available in each centre, or estimated by wingspan, when needed. Height was recorded in centimetres (cm), adjusted to one decimal place [37].

Body mass index (BMI) was calculated using the appropriate formula and data was recorded, with accuracy of one decimal place [37].

Weight for age, height for age and BMI for age were coded into z-scores, according to World Health Organization standard growth references [WHO standards (birth to 60 months) and WHO reference 2007 (61 months to 19 years)] [37]. For individuals older than 19 years, z-scores standardized to 19 years were given. In individuals older than 10 years, weight wasn't analysed based on z-score, in accordance with WHO recommendations [37].

In the adult group, patients were also classified according to the International Classification of BMI in underweight, overweight and obesity (adapted from WHO 1995, WHO 2000 and WHO 2004) [38].

When available, disease specific growth charts were used: for MPS II (weight, stature and BMI for age), MPS III (stature for age), MPS IV (weight, stature and BMI for age) and MPS VI (stature for age) [39-42].

Body composition analysis was made using the bioelectrical impedance analyser AKERN BIA-101 Anniversary® (single frequency 50Hz). Recommendations for its use with maximum accuracy were applied: discouragement of alcohol intake and physical activity in the previous 8 hours and 4 hours fasting prior to the analysis [43].

Patients with prosthetics or catheters of unknown composition were excluded from this assessment.

Resistance and reactance were recorded with accuracy of 0,1 ohms. Phase angle was calculated using the appropriate equation, like previously described in other studies, and recorded with accuracy of 0,1° [44].

III. RESISTANCE TEST

The six-minute walking test (6-MWT), a resistance test to assess ability to walk without support, unless mentioned, was performed in a 30 meter corridor, according to published guidelines [45]. The test was accomplished after bio impedance analysis. Results were recorded with an accuracy of 0.1 m.

IV. LABORATORIAL ANALYSIS

Collection of biological samples occurred in each hospital, in the same visit as the other procedures. Morning fast was recommended.

Data of each test result was displayed with an accuracy of 0.1 and presented in international standard units, except for a few, where other units are more commonly used: cholesterol (total, LDL and HDL), triglycerides, alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transferase [46].

Since reference values for some parameters were slightly different according to the diverse hospital laboratories, results are described as normal, high or low in relation to the reference values of each specific laboratory.

In the case of c-reactive protein and folic acid levels, in which results were presented as higher or lower than a certain threshold, that value was used as the result for the purpose of statistical analysis.

V. QUALITY OF HEALTH ASSESSMENT

The MPS Health Assessment Questionnaire (HAQ), an instrument to assess functional status, capabilities and performance, was applied to patients older than 14 years of age, unless cognitive impairment was present [47, 48]. In these cases, parents or legal guardians provided answers, as with children younger than 14 years of age.

The questionnaire is composed by two parts, concerning daily living activities (part I) and the need for assistance (part II). Part I consists of a series of 39 questions related to activities of daily living, grouped by domains. To each question, patients or parents should attribute, through a Likert scale type, a score according to the level of difficulty in performing the activity. It ranges from "Not difficult at all" (=0) to "Extremely difficult" (=10). When the patient cannot complete the task, a check box with the mention "Unable to do" is available to point out. Part II of the questionnaire corresponds to a series of 13 items related to categories of assistance. Each present four options according to the level of caregiver assistance needed, from "independent" to "need of complete assistance".

Respecting data analysis of Part I, it was considered the desirable age of competence acquisition the one established in the Vineland Scale [49]. When it is presented as a range (example: 2 to 3 years), maximum age was considered for the analysis. When the activities needed competences of ages higher than the present age of the individual, the associated question was not considered because it was found not appropriate or expected for that age. In the case of a patient who has already acquired the competence although it wasn't expected for that age, it was still considered for total score calculation.

For each domain of Part I, a score was obtained by the sum of all responses (varying from 0 to 11, which was credited when the "unable to do" box was marked), divided by the number of answered questions. Each category can be compared to each other since all have the same metrics, as well as total score does.

Part II score was obtained by the sum of scores (1=independent; 2=minimal assistance required; 3=moderate assistance required; 4=complete assistance required) of all 13 domains, divided by number of domains.

STATISTICAL ANALYSIS

Statistical software IBM SPSS Statistics for Windows, Version 22.0 was used to analysis [50].

Mean (M), standard deviation (SD), minimum and maximum are presented for ordinal variables. Nominal variables results are presented in absolute and relative frequencies (n and %).

The significance level of 0.05 was considered, when inferential statistics was performed [51].

The small sample size and the distribution of the subjects by the categories of nutritional intake, with a substantial proportion in only one option, determined the selection of nonparametric statistical tests [52, 53]. When it was intended to compare the scores obtained by two groups, the Mann-Whitney U test was calculated. Kruskal-Wallis test was calculated to compare three groups. In both cases, to accommodate the effects of a reduced sample and considering that some groups had a very small number of subjects, the exact p value was calculated [54]. If Kruskal-Wallis results were significant, post hoc tests were calculated using the pairwise comparisons procedure of SPSS 22.0 (Dunn-Bonferroni).

Inferential statistics was not calculated when only one group obtained more than one observation. Also, in cases where one of the three cells obtained only one observation this was not considered to the inferential analysis.

Spearman correlations (rho) were calculated in it was intended to test associations between variables. Spearman's correlation is the nonparametric alternative to Pearson's correlation [51]. The option for this non-parametric test had to do with the small sample size. The interpretation of the Spearman correlation is identical to that of Pearson, and its significance ($p<0.05$), its signal (positive or negative) and its magnitude (-1 to 1) were considered. In the interpretation of the magnitude was considered the proposal of Cohen (1988): between 0.10 and 0.29 the correlation is weak; between 0.30 and 0.49 moderate; and between 0.50 and 1.0 strong. The magnitudes of the correlations were interpreted even when this was not significant, taking this information as merely descriptive of the relationship between the variables in the study sample.

Some variables have missing data. In order to preserve as much as possible the sample size, the pairwise method was used, which consists of using all the available information for each pair of variables. In this way, the n varies in each cross between variables (correlation or test of differences). Considering that this is an exploratory study of a rare population, this method allows optimizing the data collected [55].

To test the independence of two nominal variables in the distribution, the Fisher Exact test was performed as an alternative to the Chi-square test [56].

Each MPS type group (except MPS VII) and patients submitted to ERT were compared to others as a set, in what concerns to age at diagnosis, selected nutritional status parameters (BMI z-score; phase angle; plasma pre-albumin, retinol binding protein (RBP), creatinine, essential fatty acids and vitamins D, E and A levels), nutritional intake, HAQ scores (by domain and total score) and 6-MWT results. MPS patients were also assembled according to skeleton versus central nervous system major involvement in two groups: MPS I, II, III and MPS VII (central nervous system) and MPS IV and MPS VI (skeleton). Both groups were compared for the same parameters mentioned above.

Differences were tested in the categories of nutritional intake (energy and macronutrients) in percentage of EAR (< 90%, 90 to 110%, > 110%), according to patients age. This parameter was also correlated with selected nutritional status parameters (BMI z-score; plasma pre-albumin, RBP, creatinine, essential fatty acids and vitamins D, E and A levels), HAQ scores (by domain and total score) and total distance walked in 6-MWT.

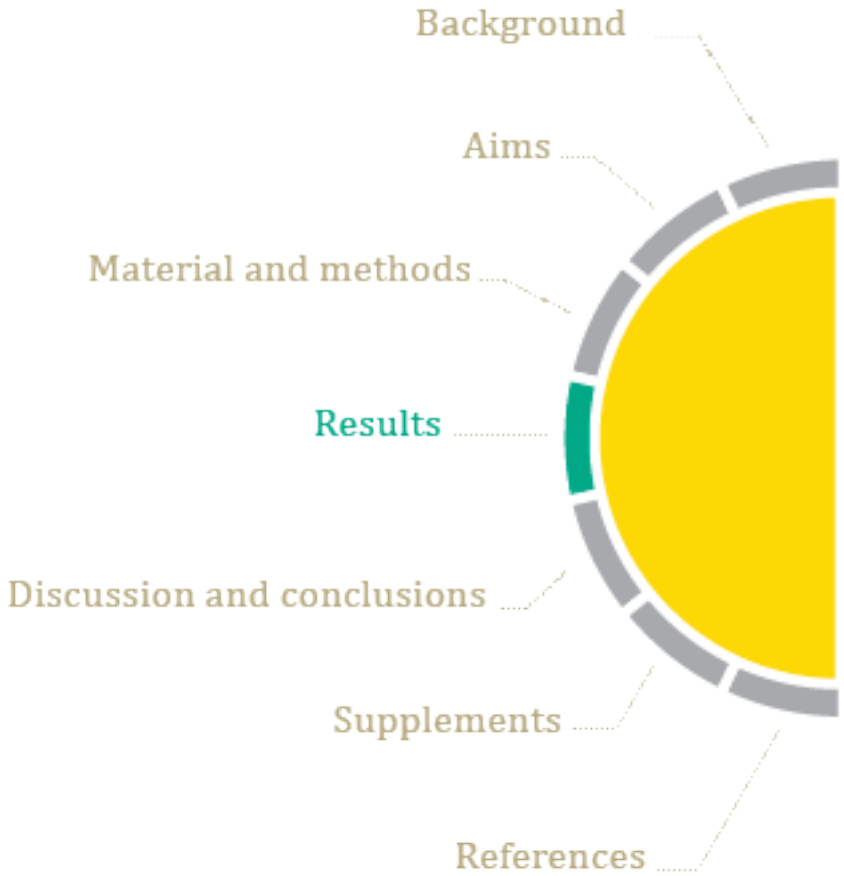
Categories of nutritional intake in percentage of EAR (< 90%, 90 to 110%, > 110%) were also correlated with results from HAQ (scores by domains and total).

ETHICAL APPROVAL

This study was submitted to the ethical committees of each Centre, according to the established rules. In one of the centres included in the study (CHSJ), it was mandatory that the investigator didn't have a personal contact with patients, so there was a need to present the protocol to the nutritionist in that team, and monitor its application.

After invitation, each patient and/or his legal guardian (parent or other) met the investigator in order to clarify all the protocol procedures and every possible doubt. Parents or other legal guardians gave a written informed consent; the patient himself, when aged more than 16-year-old and with no mental impairment, gave the consent. An appropriate consent form with a comprehensive description of the protocol, signed by the investigator, was distributed.

This investigation was also approved by the *Comissão Nacional de Proteção de Dados*.


Background
Aims
Material and methods
Results
Discussion and conclusions
Supplements
References

I. CHARACTERIZATION OF THE SAMPLE

MPS patients included in this study (31 individuals) were recruited from four national treatment centres: sixteen (51.6%) were followed in Centro Hospitalar Universitário de Coimbra, E.P.E., eight (25.8%) in Centro Hospitalar de São João - Porto, four (12.9%) in Centro Hospitalar do Porto, E.P.E. and three (9.7%) in Centro Hospitalar de Lisboa Norte.

The 31 patients, 17 males (54.8%), were aged between 1.7 and 32.7 years (mean 15.0; SD=7.5; median 14.4) (Figure 1; Table S2).

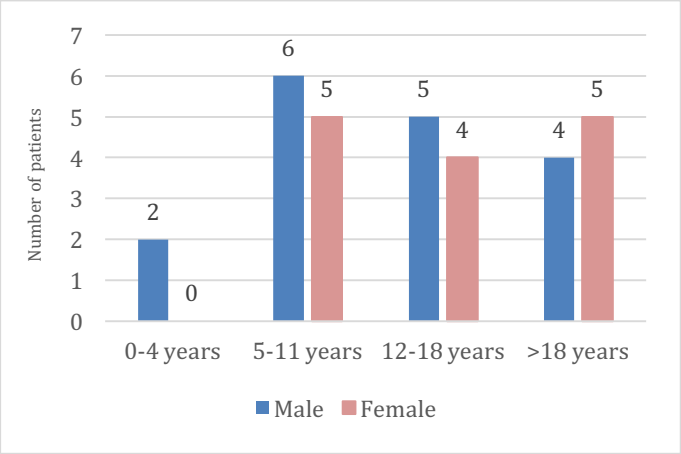


Figure 1. Characterization of the sample: distribution per gender and age group (n=31).

The sample included five MPS I (16.1%), four MPS II (12.9%), nine MPS III (29.0%; six subtype B and three subtype C), three MPS IV (9.7%), nine MPS VI (29.0%) and one MPS VII (3.2%) patients (Figure 2 and Figure 3).

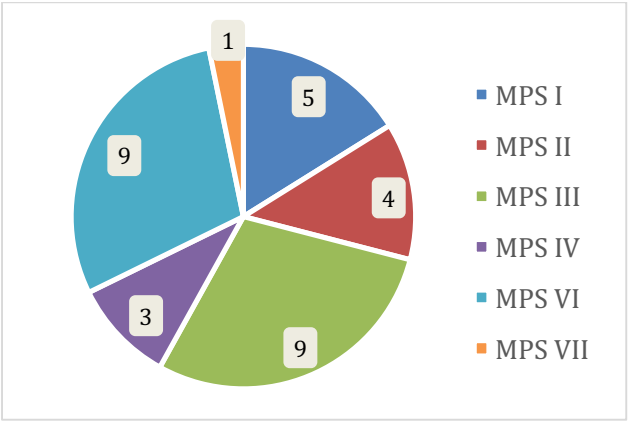


Figure 2. Characterization of the sample: number of patients per type of MPS (n=31).

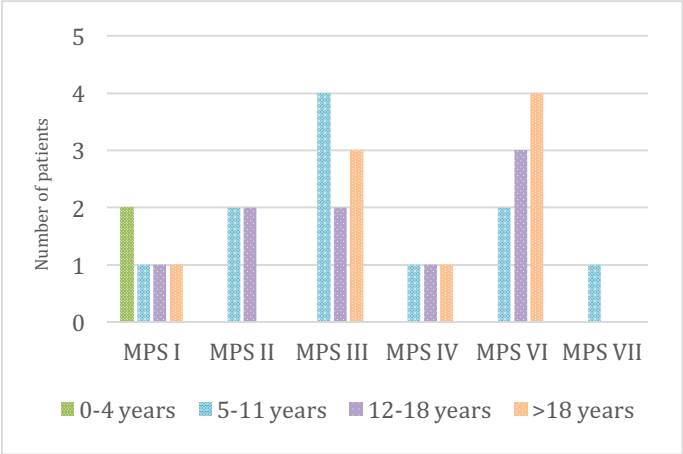


Figure 3. Characterization of the sample: type of MPS by age group (n=31).

Patients were diagnosed with MPS at a mean age of 3.2 (SD=3.0; median 2.3) years, ranging from 1 month (0.1 years) to 12.3 years (Table S3).

Diagnosis occurred earlier in the MPS VII patient (0.1 years) and in MPS I (2.2 ± 2.3 years; median 2.0) and MPS VI (2.3 ± 1.3 years; median 2.0) patient groups. MPS IV and MPS II patients were diagnosed later, at 5.5 (SD=5.3; median 5.0) and 5.7 (SD=5.7; median 2.6) years, respectively (Table S3).

MPS types (except MPS VII) were compared according to mean age at diagnosis (Figure 4). No statistical significant difference was found.

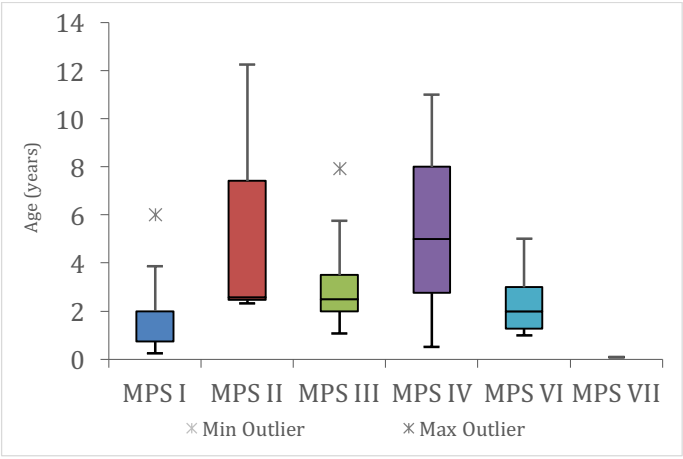


Figure 4. Characterization of the sample: age at diagnosis per type of MPS (n=31).

Twenty-six patients (83.9%) in this study were under chronic pharmacological treatment (Table S1). Anti-epileptic drugs were regularly used by ten.

Nineteen patients were under enzymatic replacement therapy (Figure 5 and Table S1).

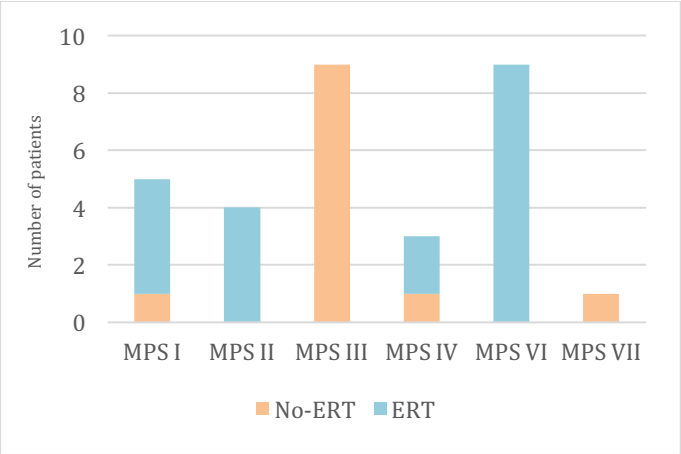


Figure 5. Characterization of the sample: enzymatic replacement therapy (ERT), per type of MPS (n=31).

Ten patients were taking nutritional supplements. Six were submitted to vitamin D supplements, isolated (MPS I_2, MPS I_5, MPS II_1, MPS VI_4 and MPS VI_6) or associated with calcium (MPS VI_7). This patient was also taking an iron supplement. Folic acid was used by two patients (MPS I_2 and MPS I_4). Three patients (MPS I_5, MPS III_1 and MPS III_7) mentioned the use of a "complete nutritional supplement" (Table S1).

II. FOOD AND NUTRITIONAL INTAKE

A. RECENT NUTRITIONAL INTAKE

Recent nutritional intake data, based upon the information collected with the 24-hour recall questionnaire, was applied in all cases.

A mean caloric intake of 65.4 Kcal/Kg/day was found (SD=29.5; median 52.5), with a minimum of 20.2 Kcal/Kg/day in patient MPS VI_5 and a maximum of 133.5 Kcal/Kg/day in patient MPS I_5. Macronutrients distribution is present in Figure 6. Additional data, concerning energy, macronutrients and micronutrients is displayed in Table S4 and Table S5 (Supplements).

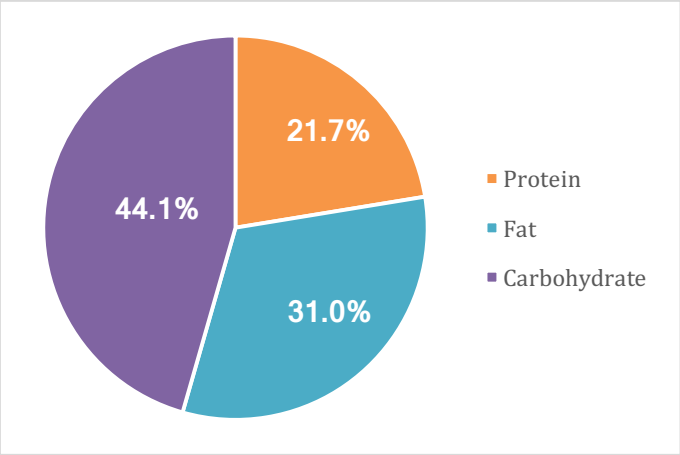


Figure 6. Recent nutritional intake: mean distribution (% of total caloric intake) of macronutrients (n=31).

B. FOOD HABITS AND USUAL NUTRITIONAL INTAKE

Twenty-five individuals (81% of the sample) answered the food-frequency questionnaire on the day nutritional assessment was done. Data is summarized in Table 2 and presented in detail in supplements section (Table S6 to Table S18).

Frequency of intake	Food / group of food	Absolute frequency (relative frequency)
Daily	Reduced fat milk	15 (60.0%)
	Yogurt	12 (48.0%)
	Vegetables soup	11 (44.0%)
Weekly	Chicken	18 (72.0%)
	Turkey, rabbit	14 (56.0%)
	Cow, pork and goatling meats	17 (68.0%)
	Ham and sausage	12 (48.0%)
	Sausages	14 (56.0%)
	Fatty fish such as sardines, mackerel, horse mackerel, salmon, etc.	14 (56.0%)
	Lean fish such as hake, pouting, gold, etc.	17 (68.0%)
	Cod	10 (40.0%)
	Olive oil	12 (48.0%)
	Butter	11 (44.0%)
	White bread or toast	11 (44.0%)
	Rice	20 (80.0%)
	Pasta like spaghetti, macaroni, etc.	20 (80.0%)
	Boiled potatoes, baked, stewed and pureed	22 (88.0%)
	Maria type crackers, water and salt or full	13 (52.0%)
	Carrots	13 (52.0%)
	Apple and pear	9 (36.0%)
	Ice tea	10 (40.0%)
Monthly	Eggs	10 (40.0%)
	Other soft drinks, fruit juices or nectars packed	7 (28.0%)
	Hamburger	11 (44.0%)
Never (or less than once a month)	Other food	10 (40.0%) - 25 (100.0%)

Table 2. Food habits (mode): absolute and relative frequencies (n=25).

Data concerning energy and macronutrients, obtained through food-frequency questionnaire results, is shown in Figure 7 and in Table S19 in supplements section.

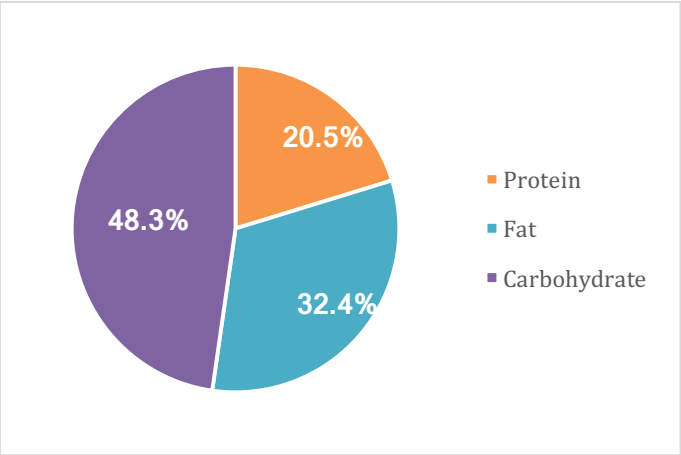


Figure 7. Usual nutritional intake: mean distribution (% of total caloric intake) of macronutrients (n=25).

A mean **caloric intake** of 77.5 Kcal/Kg/day was found (SD=38.7; median 72.6), ranging from 395.2 Kcal in a 12-year-old MPS III boy (MPS III_6) to 3910.1 Kcal in an 11- years-old MPS II boy (MPS II_4) (Table S19).

Fifty six percent of the subjects showed a lower caloric intake than recommendations. In 28.0% the intake was higher than recommended. In 4 patients (MPS I_1, MPS I_3, MPS II_2, MPS VI_2), caloric intake was considered adequate (Figure 8; Table S20 to Table S22).

Data related to energy intake adequacy per type of MPS is displayed in Figure 9 and Table *S23*.

Protein intake was considered adequate in 44.0% of the subjects, with a mean ingestion of 97.8 grams per day (Table S25). A minimum intake of 23.5g and a maximum of 160.6g were found in the same patients in whom caloric intake was the lowest and the highest, respectively MPS III_6 and MPS II_4 (Table S19).

Eight patients showed inadequate intake of this nutrient; in the total sample, adequacy was 102.6%. Inadequate intake was more prevalent in patients with 5 to 11 years of age (55.6%) and in MPS VI patients (55.6%) (Figure 8; Table S26 to Table S29).

Concerning **carbohydrates**, 64.0% of the subjects had an intake lower than recommendations (Table S25). The lowest was 44.6g and the highest was 626.6g, in the same patients mentioned above (MPS III_6 and MPS II_4, respectively) (Table S19).

Mean adequacy was 87.9% in the total sample (Table S25). Inadequate intake of carbohydrates was of 77.8% among patients aged between 12 and 18 years, and 100.0% in adults (Table S26 and Table S27). All MPS I patients and 66.7% of MPS VI showed an inadequate carbohydrate intake (Figure 8; Table S28 and Table S29).

Fiber intake ranged from 5.1 to 76.2 grams per day (mean 20.6; SD15.8) (Table S19). Nineteen patients (76.0%) showed lower intake than recommendations.

The mean adequacy of **fat intake** was 110.6 % (SD 20.0) in the total sample tested (25 patients) (Table S25 to Table S29). It was adequate in 52.0% (mean 69.9g; SD 24.5g) (Table S19). The lowest intake (11.3g) was found in MPS III_6, the boy mentioned above, which was considered adequate in percentage of the caloric intake. The highest (364.4g) was observed in a 6-year-old MPS I girl (MPS I_2). Regarding omega 3 fatty acids, 18 patients (63.8%) showed an inadequate intake, reaching the lowest mean of adequacy in MPS VI patients group. Two patients showed adequate intake of omega 6 fatty acids (MPS VI_7 and MPS VI_9), whereas 23 had an intake lower than EAR (Figure 8 and Table S25).

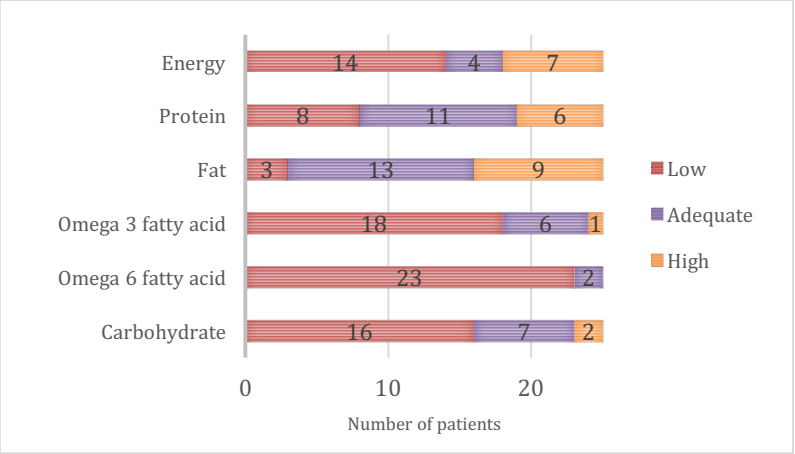


Figure 8. Usual nutritional intake in MPS patients (energy and macronutrients): number of patients consuming lower, adequate and higher than recommendations (n=25).

In Figure 9, presented next, adequacy of energy and macronutrients intake is displayed according to type of MPS. No statistical significant differences were found in energy and macronutrients intake between different groups of MPS types. More detailed data is showed in Table S24 and Table S28.

Adequacy of energy and macronutrients intake was similar in MPS patients with major central nervous system involvement (MPS I, II, III and VII) and major skeleton involvement (MPS IV and VI).

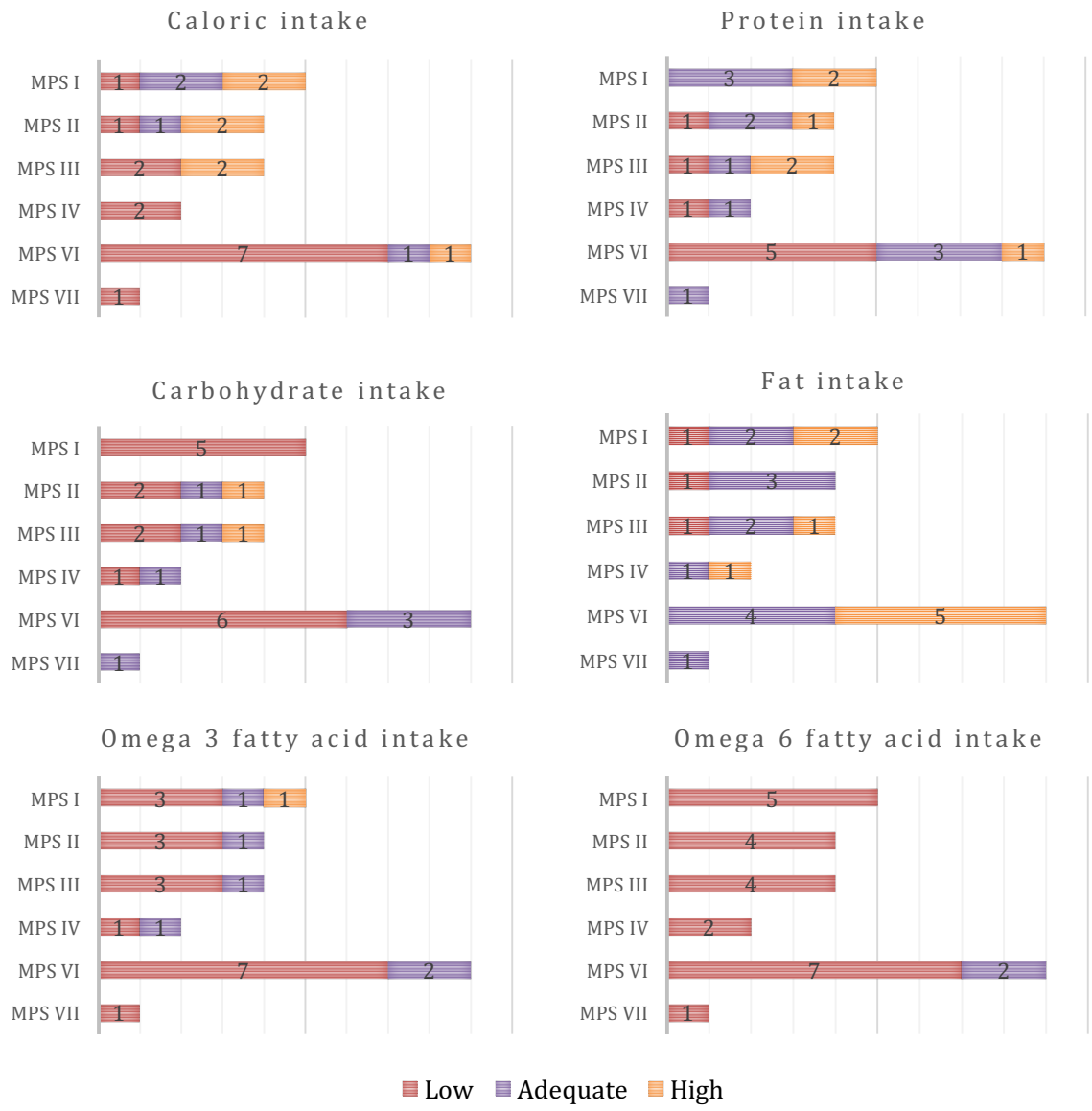


Figure 9. Usual nutritional intake in MPS patients (energy, macronutrients and essential fatty acids): number of patients consuming lower, adequate and higher than recommendations, per type of MPS (n=25).

Energy and macronutrients intake adequacy in percentage of EAR (< 90%, 90 to 110%, > 110%) did not vary with age.

Adequacy of energy and macronutrients intake was similar in patients under ERT and others.

Vitamins

Vitamins intake was higher than recommendations in the majority of patients for most vitamins, namely, vitamin A, vitamin B_1, vitamin B_3, vitamin B_6, vitamin B_{12} and vitamin C, reaching adequacies between 227.1% and 825.2% (Figure 10; Table S30 to Table S35).

Twenty patients (80.0%) showed an insufficient intake of biotin. Folate and pantothenic acid intakes were insufficient in nine patients, of whom, six had MPS VI.

One 18-year-old MPS II boy (MPS II_3) showed an adequate intake of vitamin D; the remaining 24 patients showed an insufficient intake.

Vitamin K intake was considered adequate in the MPS III_6 boy. The remainder presented insufficient intake, which contributed to a mean of adequacy of 26.3%.

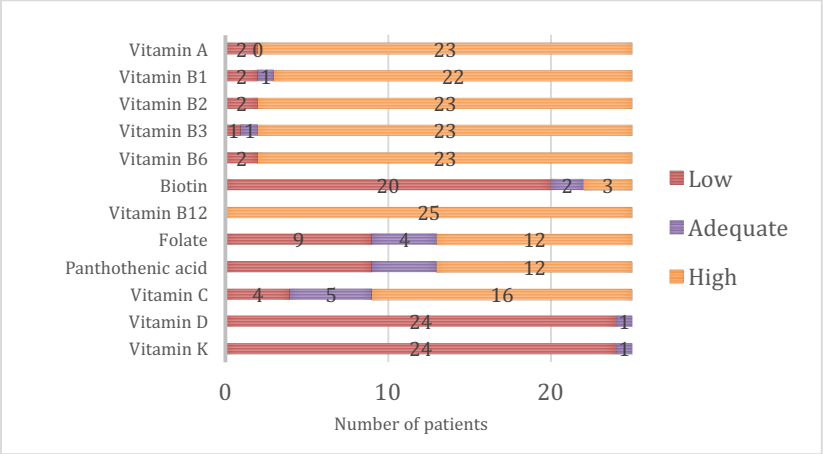


Figure 10. Usual nutritional intake in MPS patients (vitamins): number of patients consuming lower, adequate and higher than recommendations (n=25).

Minerals and oligoelements

Manganesium intake was insufficient in all 25 patients (mean of adequacy 0.2%). Seventeen patients showed an insufficient potassium intake. For the remaining minerals and oligoelements, mean adequacy was found to be between 114.6% for calcium (lowest 75.1% in 12 to 18 years) and 335.4% for copper intake. Sodium intake was lower than recommendations in two patients, one 9-year-old MPS III girl (MPS III_4) and one 12-year-old MPS III boy (MPS III_6). More data is displayed in Figure 11 and in supplements (Table S36 to Table S41).

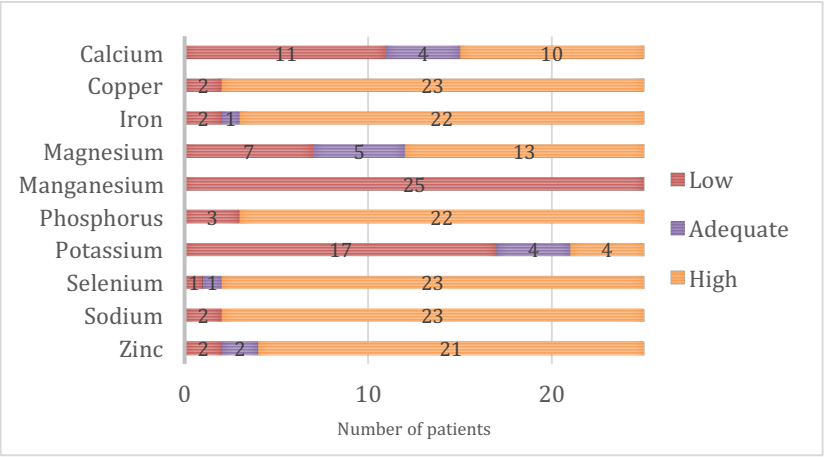


Figure 11. Usual nutritional intake in MPS patients (minerals and oligoelements): number of patients consuming lower, adequate and higher than recommendations (n=25).

III. ANTHROPOMETRICS AND BODY COMPOSITION

Patients' **weight** ranged from 12.3 to 56.8 Kg (mean 30.2; SD 10.3; median 29.5). Weight z-score extended from -1.7 to 2.6 (Table S42). Weight z-score was the lowest in MPS VI (-1.0) and the highest in MPS III (1.5) patients group (Table S42 and Figure 12).

Mean weight z-scores in the different age groups were: 0.0 (SD=0.9) under 4 years of age and -0.1 (SD=1.8) from 5 to 11 years. A minimum weight z-score of -2.8 was found in a 6-year-old female patient with MPS I (MPS I_2). A 5-year-old boy with MPS III (MPS III_8) presented the maximum weight z-score of 1.8 (Table S43).

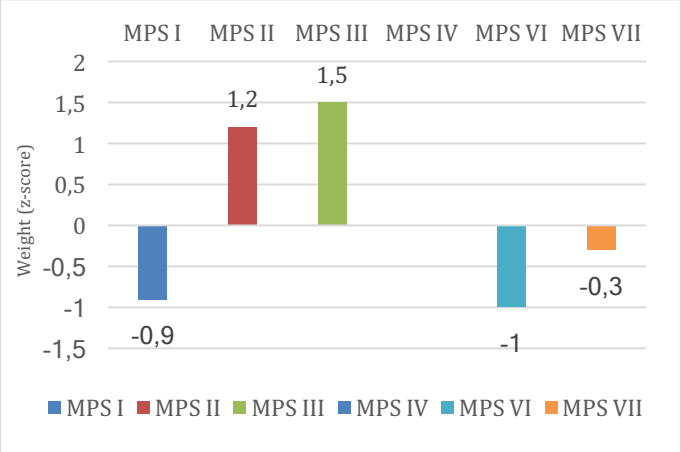


Figure 12. Anthropometrics: weight, in z-score, according to MPS type (n=9: three MPS I; one MPS II; two MPS III; two MPS VI; one MPS VII).

Patients' **height** ranged from 83.3 to 170.0 cm (mean 124.2 ± 20.5 cm) and z-score from -10.1 to 1.5 (Table S44). Minimum mean height z-score was found in MPS IV patients group (-7.3) (Figure 13). At the youngest group, mean z-score was -0.3 (SD=0.0), from 5 to 11 years, -1.7 (SD=2.7), from 12 to 18 years, -5.1 (SD=2.0), and in the oldest group, -5.6 (SD=3.9) (Table S45).

A strong negative correlation was found between height z-score and age (rho=-0.555; p=0.001) (Figure 14). The lowest height z-score (-10.1) was detected in a 32-year-old MPS IV woman (MPS IV_3).

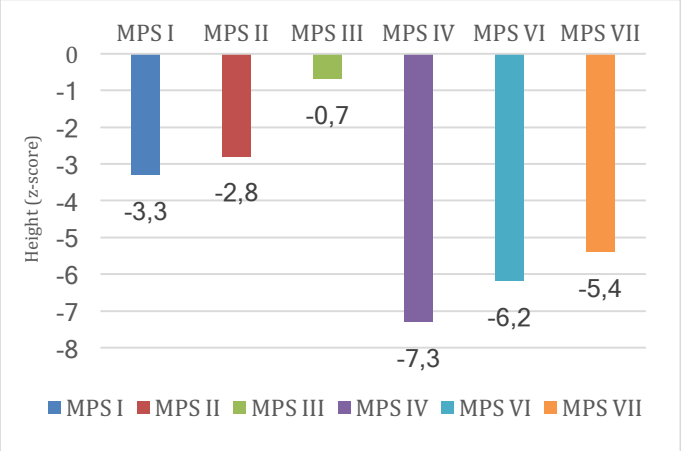


Figure 13. Anthropometrics: height, in z-score, according to type of MPS (n=30: five MPS I; four MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII).

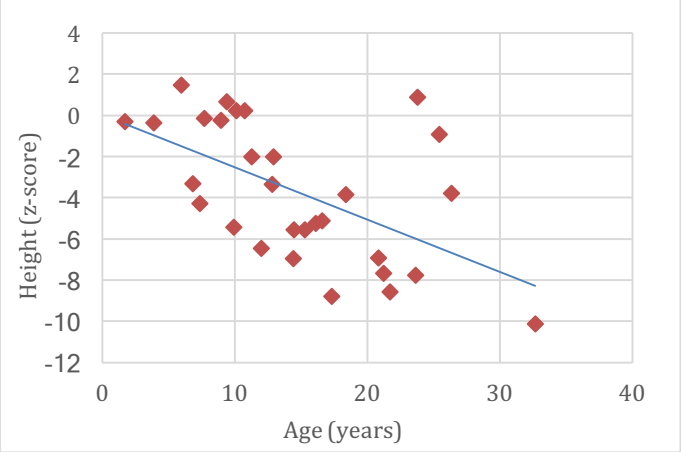


Figure 14. Anthropometrics: height, in z-score, according to age (n=30: five MPS I; four MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII). A trend line is displayed in blue.

Concerning **BMI**, the sample showed a mean value of 19.3 Kg/m^2 (SD=3.9), ranging from 13.0 to 28.8 Kg/m^2. Mean BMI z-score was 0.0 (SD=1.6), varying from -4.6 in a 25-year-old MPS III man (MPS III$_7$) to 3.4 in the 9-year-old boy with MPS VII (MPS VII$_1$) (Table S46 and Table S47).

A moderate negative correlation was detected between age and BMI z-score (rho=-0.353) (Figure 15).

The lowest mean BMI z-score was found in MPS I group (-1.1) and the highest, in the patient with MPS VII (3.4) (Figure 16 and Table S46).

BMI z-score was similar between groups with low, adequate or higher intakes of energy and macronutrients.

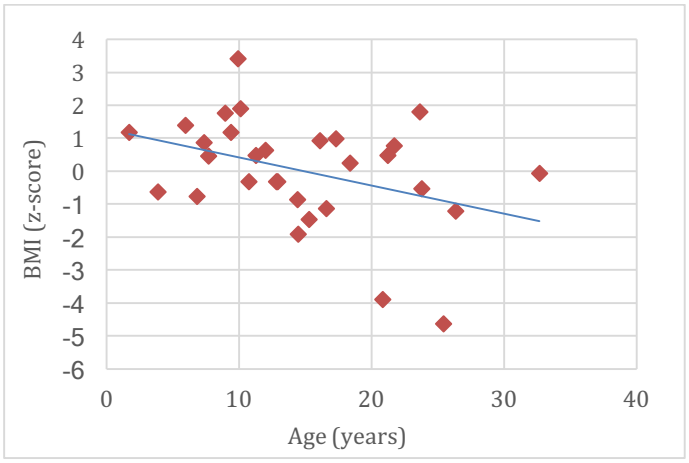


Figure 15. Anthropometrics: BMI, in z-score, according to age (n=30: five MPS I; four MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII). A trend line is displayed in blue.

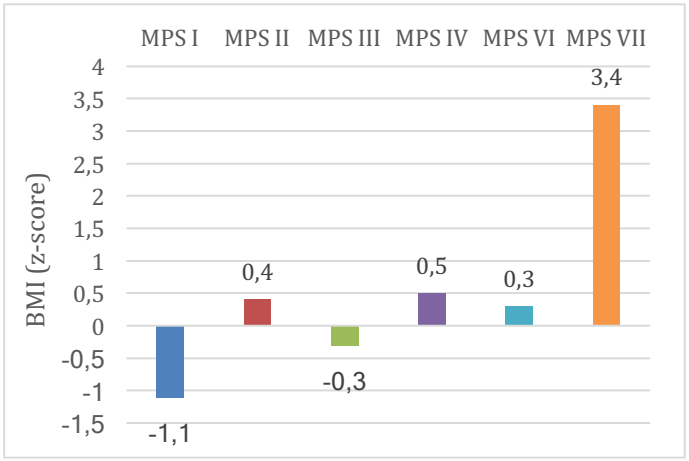


Figure 16. Anthropometrics: BMI, in z-score, per MPS type (n=30: five MPS I; four MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII).

The great majority of patients (73.3%) showed normal growth according with World Health Organization BMI z-score tables. In two individuals (MPS I_5 and MPS III_7), a status of severe thinness was found (6.7%). Six patients (MPS II_2, MPS III_3, MPS III_4, MPS III_8, MPS VI_5 and MPS VII_1) presented overweight, one being obese (MPS VII_1) (Figure 17 and Table 3). When comparing with disease specific BMI growth curves (available for MPS II, MPS III and MPS IV), only patient MPS III_3 presents overweight.

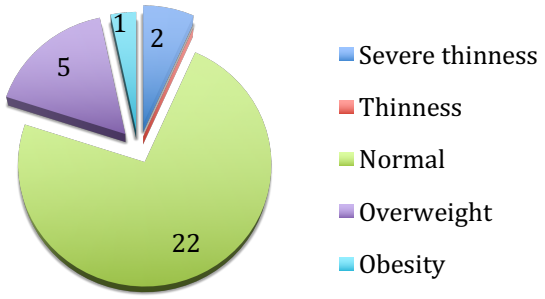


Figure 17. Distribution of individuals (number of patients), according to BMI and WHO z-score tables (n=30: five MPS I; four MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII).

Group of disease	Severe thinness	Thinness	Normal	Overweight	Obesity
MPS I	1	0	4	0	0
MPS II	0	0	3	1	0
MPS III	1	0	5	3	0
MPS IV	0	0	3	0	0
MPS VI	0	0	7	1	0
MPS VII	0	0	0	0	1
Total	2 (6.7%)	0 (0.0%)	22 (73.3%)	5 (16.7%)	1 (3.3%)

Table 3. Anthropometrics: classification of nutritional status according to BMI (n=30: five MPS I; four MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII).

Body composition was evaluated by bioimpedance in 21 patients: one MPS I, four MPS $II_{(1-4)}$, seven MPS $III_{(1-3;\ 5;\ 7-9)}$, three MPS $IV_{(1-3)}$ and six MPS $VI_{(2;\ 4-8)}$. Body fat, body lean mass and total body water were calculated in 18 patients. Minimum mean body fat mass was displayed in MPS VI group of patients (16.6%). Mean values of total body water varied between 62.4 and 65.3% (Figure 18). Additional data is presented in Table S48.

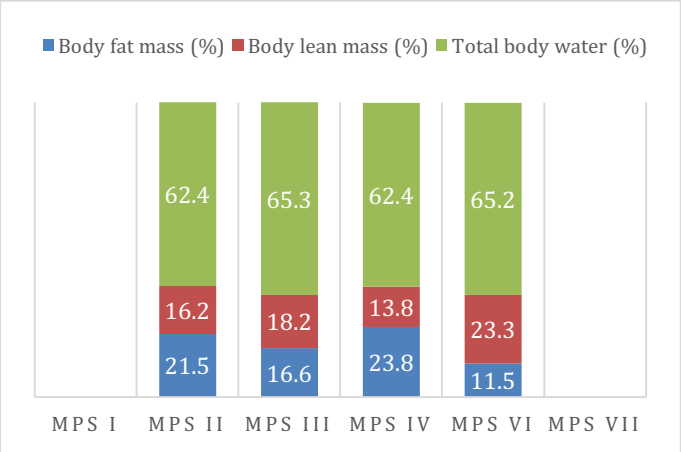


Figure 18. Body composition: mean values of body fat mass, body lean mass and total body water, in MPS types II (four patients), III (six), IV (three) and VI (five) (n=18).

Phase-angle results (n=21) varied from a minimum of 3.0° in MPS III_2 patient, to a maximum of 6.2° in MPS VI_2, presenting a mean value of 4.6° (SD=0.8) (Figure 19 and Table S49).

A correlation between age and phase angle was found to be positive and moderate (rho=0.353).

No statistical significant difference in phase angle between groups consuming lower, adequate or higher amounts of energy and macronutrients than recommendations was found.

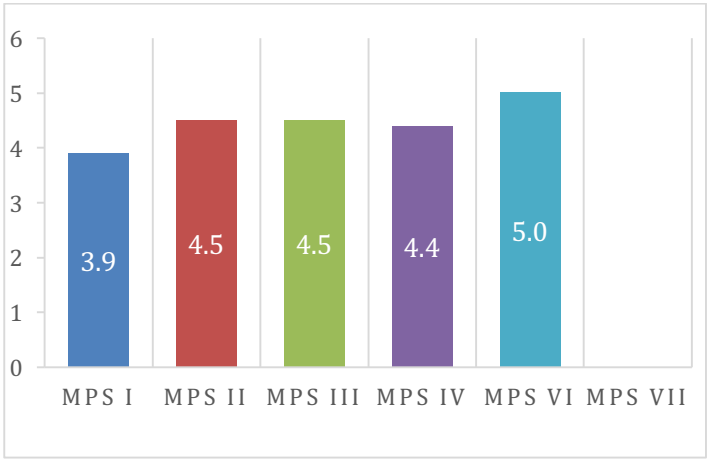


Figure 19. Phase angle: mean values (°), in MPS types I (one patient), II (four), III (seven), IV (three) and VI (six) (n=21).

IV. RESISTANCE TEST

Sixteen patients (51.6%) performed the 6-minute walking test. Twelve were unable to walk and in three cases, the test was not feasible due to structural conditions of the hospital building.

The patients who completed the test walked a mean distance of 304.6 meters (SD=147.1 m; 26.9 to 660.0 meters) (Figure 20 and Table S50). MPS type II patients showed significantly higher results in this test (U=0.0; p=0.026) (Figure 20).

No statistical differences were found between MPS group with major central nervous system involvement and that with major skeleton involvement, concerning 6-MWT achievements.

No correlation was found between age and 6-MWT results.

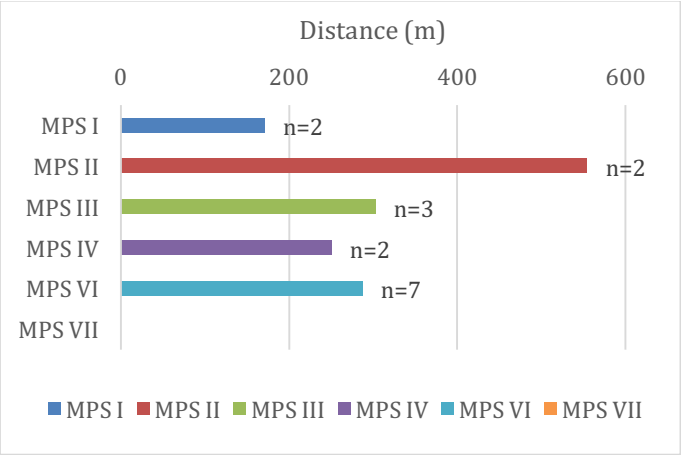


Figure 20. Total distance walked (in meters): mean values for each type of MPS (n=16).

V. LABORATORIAL DATA

Plasma proteins

Plasma **total protein** was analysed in 27 patients (Table S51 and Figure 21). Twenty-five (92.6%) presented normal values. Two adults, MPS III_1 and MPS III_7, showed lower results (both 64.0 g/L).

All patients were submitted to plasma **albumin** analysis. Twenty-seven had normal values (87.1%), whereas two individuals, MPS I_5 and MPS III_2, showed hypoalbuminemia (34.3 and 36.6g/L) and in other two values were higher than reference (51.0 and 52.0 g/L).

Plasma **ferritin** was normal in all patients (n=30) submitted to analysis.

Normal values of **pre-albumin** were detected in 11 of 27 patients (40.7%). Levels were low in the remaining 16 patients. One of the patients with low pre-albumin (172 mg/L) was MPS I_5, who was taking a "complete nutritional supplement". Plasma pre-albumin was significantly higher in MPS III patients (U=21.0; p=0.012) (Figure 22). Plasma pre-albumin levels were lower (U=35.0; p=0.028) in patients submitted to ERT, when compared to patients not performing ERT (Figure 23).

Eighteen of 24 individuals showed low levels of **RBP**, from 0.0 to 38.4 mg/L. The lowest value was detected in the only MPS VII patient; levels were above 10.0 mg/L in the other 17 patients. The patient mentioned above (MPS I_5), showed low plasma RBP (32.0 mg/L). MPS III patients showed significantly higher RBP levels than other MPS patients (U=15.0; p=0.018). MPS VI patients showed lower results than other patients (U=27.5; p=0.024) (Figure 24).

Transferrin was normal in 21 of 23 individuals. One 14-year-old boy (MPS VI_3) and one adult man with MPS VI (MPS VI_6) presented low and elevated values, respectively.

C-reactive protein was normal in 30 of 31 patients (96.8%). One man aged 26 years (MPS III_9) presented a value above the reference range (11.1 nmol/L).

No statistical correlations (weak negative) were found between age and plasma pre-albumin, retinol binding protein, and creatinine levels.

No statistical significant difference was found between groups consuming lower, adequate or higher amounts of energy and macronutrients than recommendations in what concerns plasma pre-albumin, retinol binding protein and creatinine.

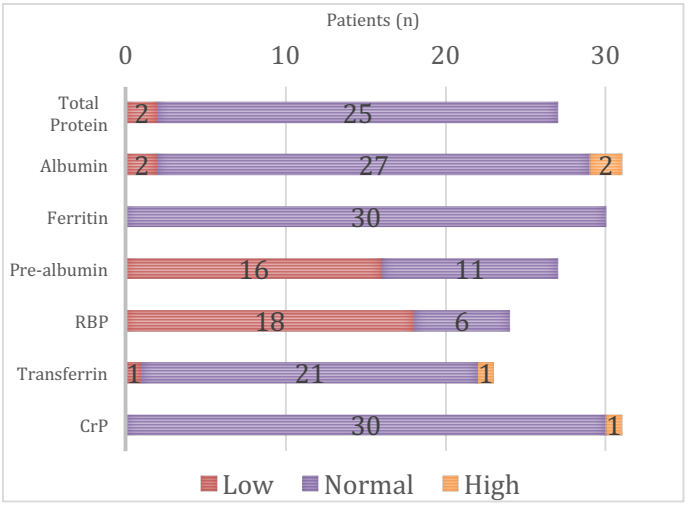


Figure 21. Plasma proteins - classification according to reference values (RBP=retinol binding protein; CrP=c-reactive protein; labels correspond to the number of patients).

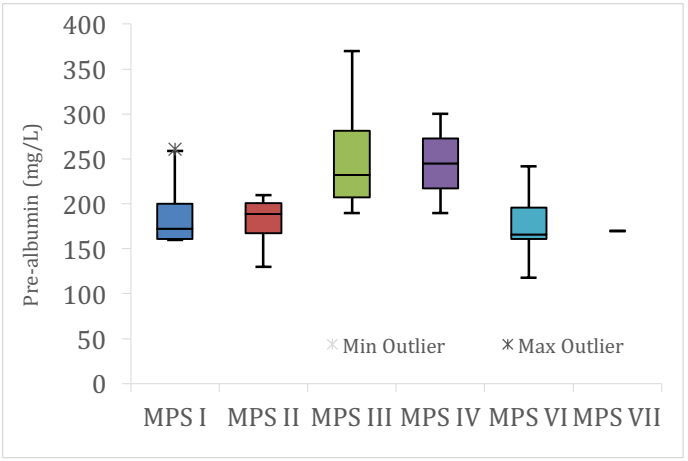


Figure 22. Plasma proteins – pre-albumin levels (mg/L), according to MPS type (n= 27: five MPS I; four MPS II; six MPS III; two MPS IV; nine MPS VI; one MPS VII).

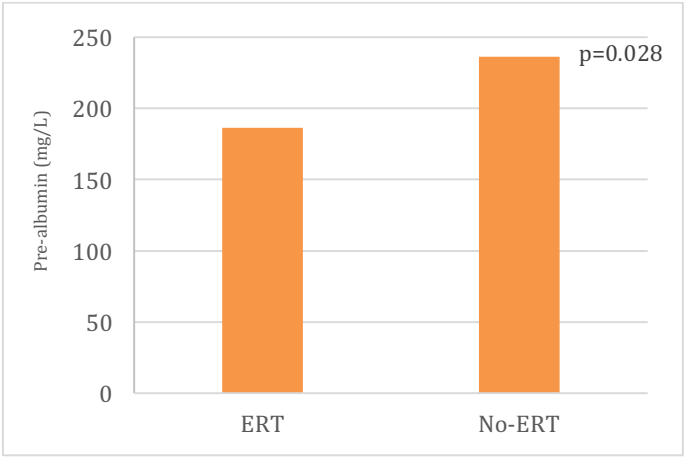


Figure 23. Plasma pre-albumin levels of ERT patients (n=19), when compared to the group not performing this treatment (no-ERT, n=8).

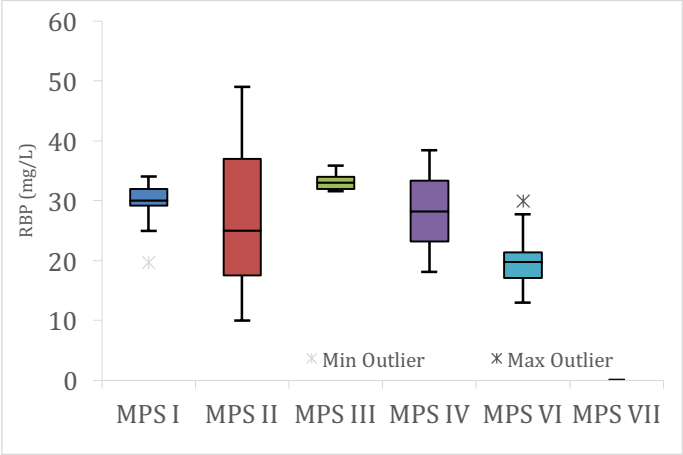


Figure 24. Plasma proteins – RBP levels (mg/L), according to MPS type (n= 24: five MPS I; three MPS II; five MPS III; two MPS IV; eight MPS VI; one MPS VII).

Lipids

Lipid profile analysis was performed in all (31) patients (Table S52 and Figure 25).

Total cholesterol was normal in 30 individuals (96.8%). The remaining patient, a 14-year-old boy (MPS VI_3) showed a value of 208.0 mg/dL.

LDL-cholesterol was normal in 28 (90.3%). In 3 patients, it was above reference range (9.7%), with a maximum of 151.0 mg/dL detected in MPS VI_3, the boy with high total cholesterol indicated above.

HDL-cholesterol was normal in 14 (45.2%) and low in 15 individuals (48.4%), reaching a minimum of 10.0 mg/dL in a 9-year-old girl (MPS III_2). In the remaining two patients (6.5%), MPS I_4 and MPS II_1, high values of HDL-cholesterol were disclosed (76.0 and 66.0 mg/dL, respectively).

Normal plasma **triglycerides** were detected in 29 individuals (93.5%). MPS III_2 showed hypertriglyceridemia (159.0 mg/dL) and MPS II_1 presented a low value (36.0mg/dL). These patients had low and high HDL-cholesterol, respectively.

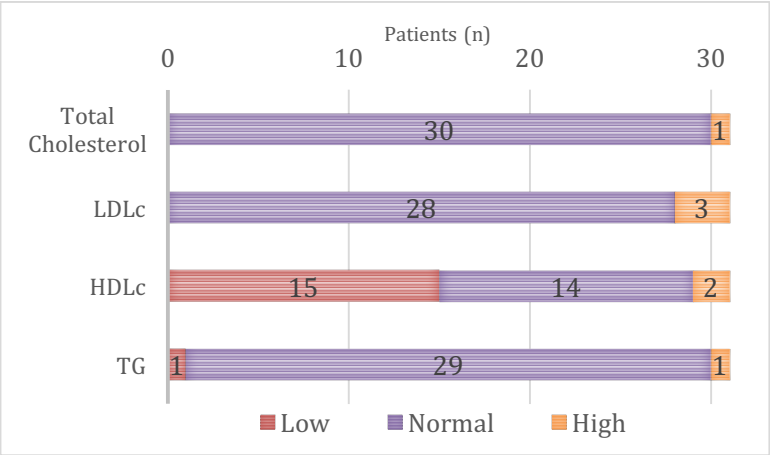


Figure 25. Lipid profile - classification according to reference values (n=31) (LDLc=LDL cholesterol; HDLc=HDL cholesterol; TG=triglycerides; labels correspond to the number of patients).

Essential fatty acids

Plasma essential fatty acids levels were evaluated in 12 patients (Table S53 and Figure 26). None was taking a specific essential fatty acids supplement.

Araquidonic acid level was normal in 8 individuals (66.7%). Four presented high values: from 270.0 µg/mL in MPS IV_1 to 369.0 µg/mL in MPS VI_3 patients. Plasma araquidonic acid levels were significantly higher in MPS VI patients (U=2.0; p=0.036) (Figure 27).

Docosahexaenoic acid was normal in 10 individuals (83.3%) and low in two (16.7%), MPS III_2 and MPS VI_1 with, respectively, 15.3 and 21.5 µg/mL.

All patients showed normal values for **eicosapentaenoic acid**.

Correlations between age and plasma araquidonic (rho=0.308) and docosahexaenoic (rho=0.336) acids were positive and moderate.

Essential fatty acids levels did not show any statistical significant difference between groups consuming different amounts of energy and macronutrients.

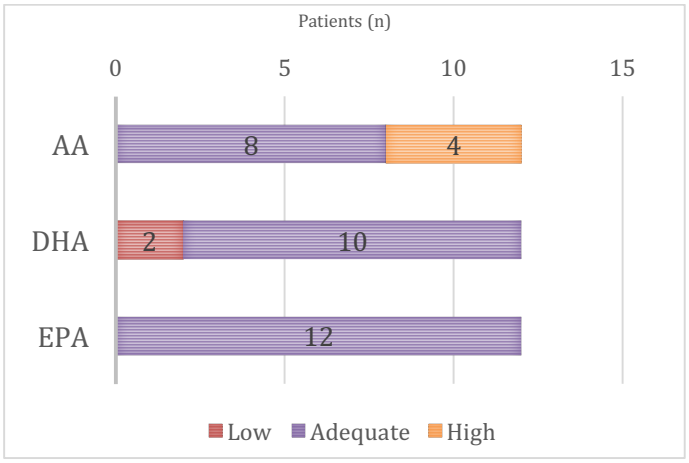


Figure 26. Plasma essential fatty acids levels in 12 MPS patients (three MPS I; four MPS III; two MPS IV; three MPS VI) - classification according to reference values (labels correspond to the number of patients) (AA=araquidonic acid; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid).

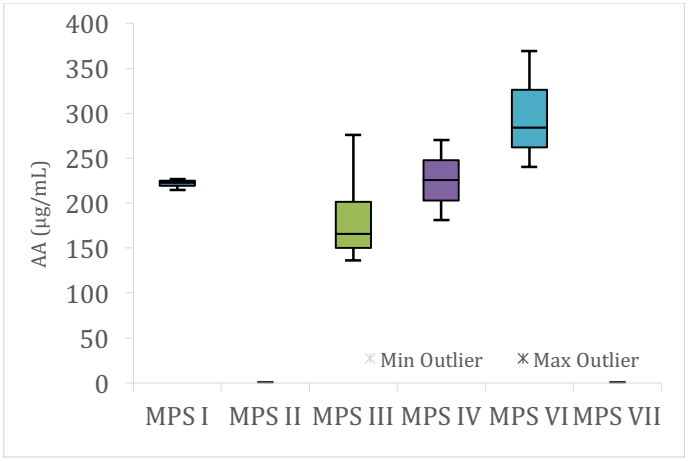


Figure 27. Plasma essential fatty acids in 12 patients – araquidonic acid (AA) levels (µg/mL) per type of MPS (three MPS I; four MPS III; two MPS IV; three MPS VI).

Vitamins

Data from plasma vitamins levels, analysed in most patients, is displayed in Table S54. Results of vitamin D analysis are disclosed in the phosphocalcium metabolism section.

Folic acid plasma levels, evaluated in 20 patients (Figure 28), were normal in 17 (85.0%). Two showed high values (45.0 and 54.0 nmol/L) and one (MPS VI_4) displayed values lower than local references (4.0 nmol/L). The patient taking folic acid supplement was not submitted to this analysis.

Vitamin B_{12} levels were adequate in 19 of 30 patients (63.3%) whereas the remaining 11 individuals presented plasma levels higher than normal, ranging from 542.0 to 884.0 pmol/L (maximum in patient MPS III_2) (Figure 28). No specific supplementation was being taken by any of the participants; two patients taking complete nutritional supplements (MPS I_5 and MPS III_7) presented two of the highest levels.

Vitamin A was considered adequate in 17 of 23 patients (73.9%) (Figure 28). Six individuals showed low levels. None of these were taking vitamin A supplementation. A minimum of 0.4 µmol/L was found in a 26-year-old woman (MPS VI_4). The MPS III group showed significant higher vitamin A levels than the remaining subjects (U=21.5; p=0.019), whereas MPS VI patients showed lower significant results than others (U=12.5; p=0.001) (Figure 29). Vitamin A levels were lower in ERT patients group (p=0.02), than in patients not under enzymatic therapy.

Vitamin E was normal in 13 of 22 individuals (59.1%), whereas 6 presented low levels (min. 13.9 µmol/L in patient MPS III_7) (Figure 28). These six are not the same as those with low vitamin A levels. Three patients had elevated values, even without nutritional supplements (max. 38.2 µmol/L in MPS IV_1 patient). Vitamin E levels were significantly higher in MPS IV patients (U=7.0; p=0.040) and significantly lower in MPS VI patients (U=19.0; p=0.016) (Figure 30). A moderate negative correlation was detected between age and vitamin E levels (rho=-0.385) (Figure 31).

Vitamin A and vitamin E levels were similar between groups with low, adequate and higher intakes of energy and macronutrients than recommendations.

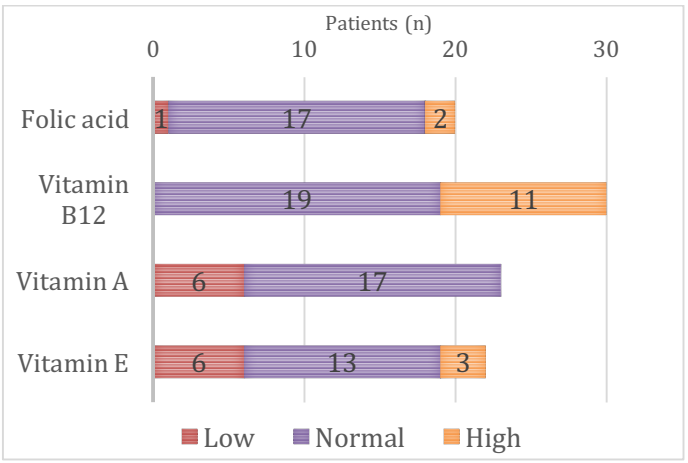


Figure 28. Plasma vitamins - classification according to reference values. (labels correspond to the number of patients)*

(*) Vitamin D – in the phosphocalcium metabolism section

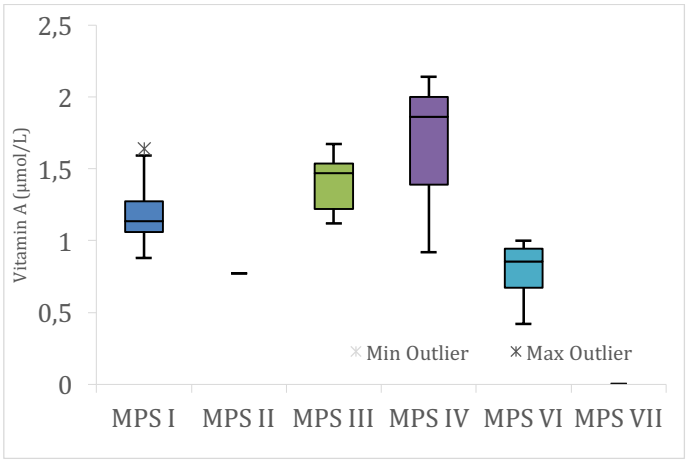


Figure 29. Plasma vitamins - vitamin A levels (µmol/L), per type of MPS (n=23: four MPS I; one MPS II; seven MPS III; three MPS IV; eight MPS VI).

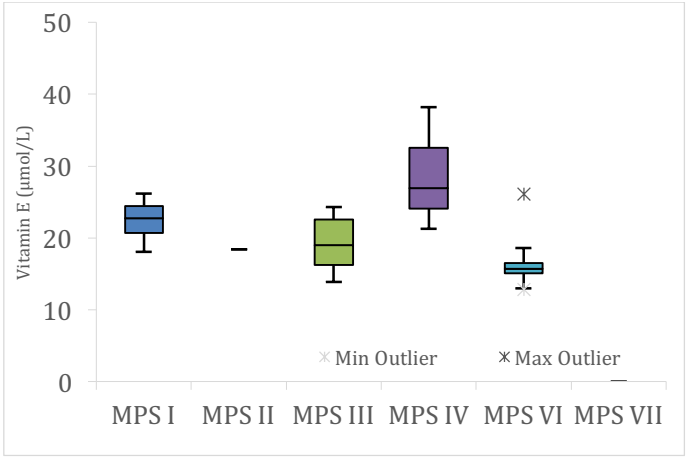


Figure 30. Plasma vitamins - vitamin E levels (µmol/L), per type of MPS (n=22: four MPS I; one MPS II; seven MPS III; three MPS IV; seven MPS VI).

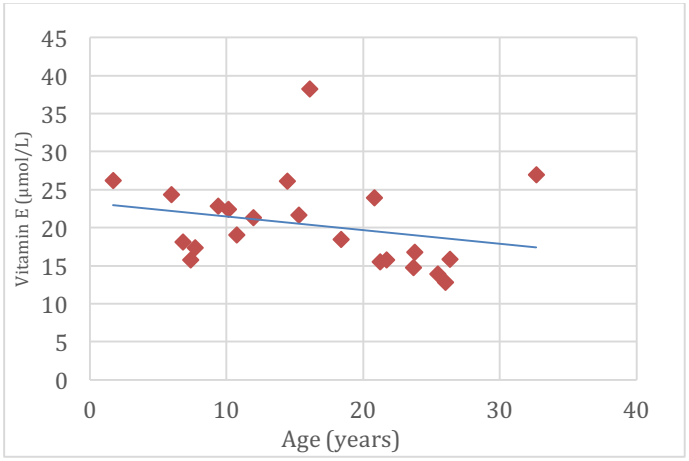


Figure 31. Plasma vitamins - vitamin E (µmol/L), according to age (n=22: four MPS I; one MPS II; seven MPS III; three MPS IV; seven MPS VI). A trend line is displayed in blue.

Minerals

Data concerning plasma concentration of minerals in patients is depicted in Table S55. Adequacy of levels is displayed in Figure 32. Results of calcium and phosphorus analysis are disclosed in the phosphocalcium metabolism section.

Twenty-one in 27 patients (77.8%) showed normal plasma **magnesium** results. Four individuals had hypomagnesemia: one of them (MPS III_7) presented the minimum of 0.70 mmol/L. Two patients had high values: MPS II_4 (0.86 mmol/L) and MPS I_5 (1.03 mmol/L). The later was taking a complete nutritional supplement.

Eleven individuals were analysed for plasma **selenium**. Normal values were detected in eight (72.7%). The other showed low values, with a minimum of 0.10 mmol/L in MPS III_8, a 5-year-old boy. Selenium was present in the complete nutritional supplement of patient MPS I_5, who displayed normal levels.

Zinc levels were normal in 23 of 24 patients (95.8%). One 18-year-old male (MPS II_3) showed a low plasma concentration of 9.5 µmol/L.

Twenty-four of 26 analysed patients showed normal results for **potassium** in plasma (92.3%). Hyperkaliaemia was found in two individuals: 5.6 mmol/L in MPS III_4 and 6.6 mmol/L in MPS II_2.

Plasma **sodium** was normal in 24 of 28 patients (85.7%). Four presented hyponatremia: a minimum of 129.0 mmol/L was detected in MPS III_7 patient.

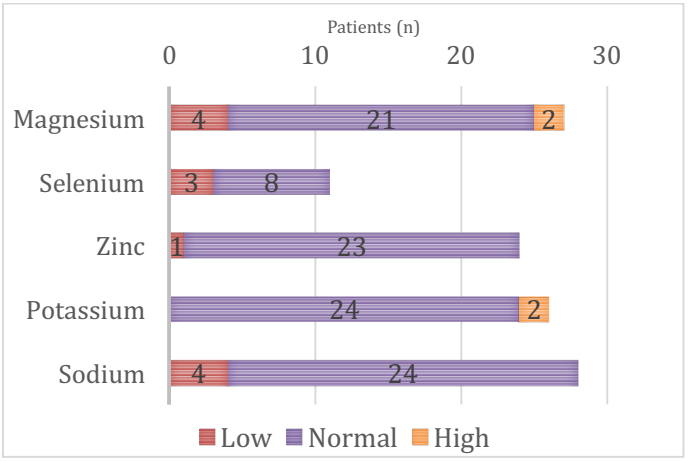


Figure 32. Plasma minerals - classification according to reference values. (labels correspond to the number of patients)

Liver function

Alanine aminotransferase (ALT) was normal in 24 of 31 patients (77.4%) (Figure 33). Seven (22.6%) presented high values, with a maximum of 79.0 IU/L found in MPS III_2 girl.

Twenty-three of 29 (79.3%) patients presented normal **aspartate aminotransferase** (AST) activity (Figure 33). Elevated values were detected in six patients (20.7%), with a maximum of 95.2 IU/L, in the patient mentioned above (MPS III_2).

Normal activity of **gamma-glutamyl transferase** was found in 18 of 28 patients (64.3%) (Figure 33). In seven patients' results, lower values were found (25.0%). Three patients (10.7%) showed results higher than references; the highest (952.1 IU/L) was detected in the 25-year-old MPS III_7 patient.

Detailed data of liver function is shown in Table S56.

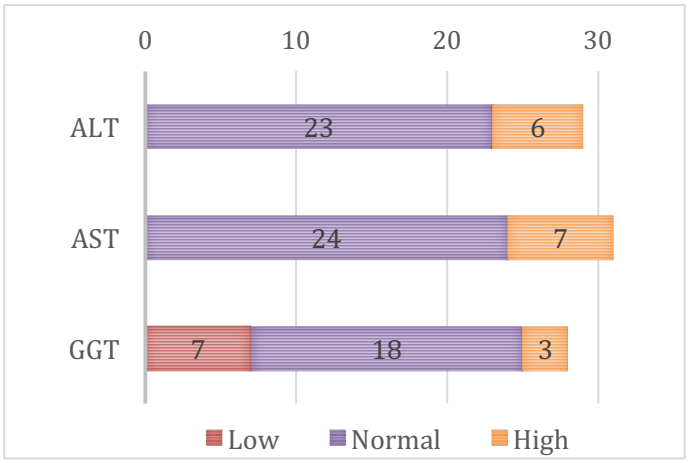


Figure 33. Liver function: classification according to reference values
(ALT=alanine transaminase; AST=aspartate transaminase; GGT=gamma-glutamyl transferase)
(labels correspond to the number of patients)

Renal function

Plasma urea and creatinine were analysed in all (31) patients (Table S57).

Urea was normal in 28 (90.3%) and low in one patient (1.1 mmol/L) (Figure 34). Two MPS I patients ($MPSI_1$ and MPS I_2) showed plasma concentrations of urea higher than reference values, 8.1 and 6.6 mmol/L, respectively.

Twenty-four patients (77,4%) presented low levels of plasma **creatinine** (minimum 12.0 µmol/L) (Figure 34). The other had normal values.

Results of calciuria and tubular phosphorus reabsorption rate analysis are disclosed later in phosphocalcium metabolism section.

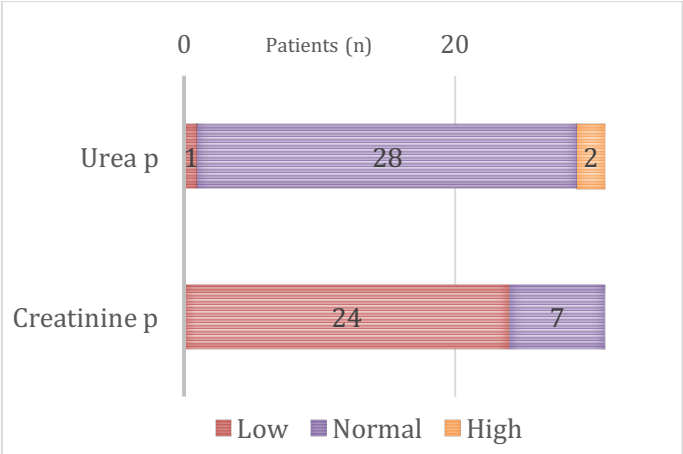


Figure 34. Renal function: classification according to reference values (n=31). (labels correspond to the number of patients)

Total blood count

All individuals (31) were tested for complete haemoglobin, leucocytes and platelets. Lymphocytes were assessed in 27 (Table S58).

Haemoglobin was normal in twenty-six (83.9%), whereas five patients showed low levels, ranging from 113.0 to 126.0 g/L (Figure 35). The lowest value was found in a 9-year-old female patient (MPS III_2). A woman (MPS VI_7) with normal levels of haemoglobin was taking an iron supplement.

Twenty-seven subjects (87.1%) presented normal **leucocytes** count (Figure 35). Two (MPSIII_7 and MPS VI_4) displayed leucopoenia presenting a sum of 2.9 and 3.6 $x10^3/\mu L$, respectively. In other two, high numbers of these cells were detected, specifically 11.8 $x10^3/\mu L$ in MPS VI_9 and 11.5 $x10^3/\mu L$ in the youngest patient, MPS I_1.

Total count of **lymphocytes** was normal in 21 of 27 patients (87.1%) (Figure 35). MPS III_8 showed a low level of 3.1 $x10^3/\mu L$.

Normal **platelets** count was detected in 22 of 31 patients (71.0%). Thrombocytopenia was found in 7 patients (22.6%), with a minimum of 78.0 $x10^3/\mu L$ in the MPS III_9 patient Thrombocytosis was present in two patients (510.0 in MPS IV_3 and 562.0 $x10^3/\mu L$ in MPS I_1). (Figure 35).

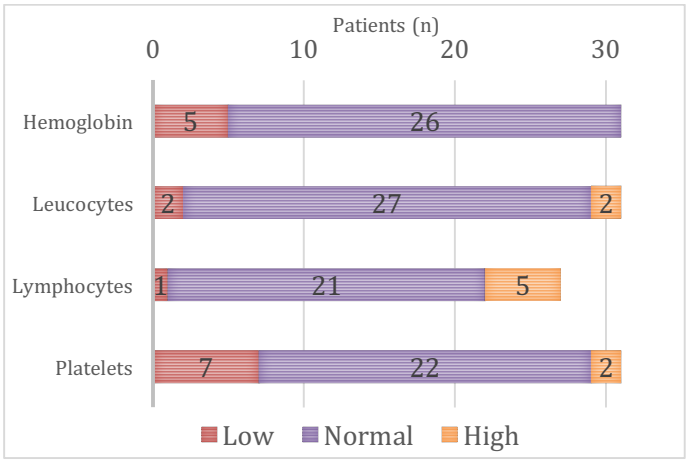


Figure 35. Total blood count: classification according to reference values. (labels correspond to the number of patients)

Phosphocalcium metabolism

Plasma **calcium** and **phosphorus** were normal in 25 (86.2%) and 24 (82.8%) of 29 patients, respectively (Figure 36). Two individuals, MPS II_2 and MPS III_9 showed low plasma calcium (2.07 mmol/L and 2.10 mmol/L, respectively). Hypercalcemia was detected in two: 2.53 mmol/L in MPS II_1, who was under vitamin D supplementation and 2.55 mmol/L in MPS I_4. Hyperphosphoremia was found in five patients, with a maximum of 1.68 mmol/L in MPS II_3 (Figure 36). None were taking nutritional supplements.

Vitamin D (25-hydroxy-cholecalciferol) plasma levels, evaluated in all (31) patients, ranged from 10.0 to 86.6 nmol/L. Non-adequate values were detected in 27 individuals (87.1%), 15 of whom presented a status of vitamin deficiency (48.4% of the total) (Figure 36 and Figure 37). Four patients presented normal vitamin D status: the youngest included (a two-year-old, MPS I_1), two other who were taking vitamin D supplements (MPS VI_6 and MPS I_5) and the one under a homeopathic supplement of unknown composition (MPS IV_3). Plasma vitamin D levels were lower in the group of patients with protein adequate intake [H(2)=6.806; p=0.033)], as compared to the groups consuming lower or higher amounts of this macronutrient.

Alkaline phosphatase activity was normal in 21 of 27 cases (77.8%) (Figure 36). Hypophosphatasemia (minimum of 1.6 µkat/L in MPS $IV_{2)}$, and hyperphosphatasemia (maximum of 2.5 µkat/L in MPS III_9) were found in three patients each.

Nineteen of the 23 (83.0%) individuals submitted to **parathormone** analysis showed normal values (Figure 36). Four presented high parathormone levels, specifically: $MPSII_1$ (a boy with vitamin D deficiency), MPS VI_4 (a woman with insufficient levels of vitamin D) (both under

supplementation), MPS I_4 (a 3-year-old boy with vitamin D insufficiency) and MPS I_3 (a girl with vitamin D deficiency). The first two were taking supplements.

Urinary calcium / creatinine was normal in all patients analysed (18), except for MPS I_5, who was taking a complete nutritional supplement and showed a high ratio (0.89) (Figure 36).

Tubular reabsorption of phosphate was normal in all 17 patients investigated for that parameter (Figure 36).

Data concerning phosphocalcium metabolism indicators is presented in Table S59.

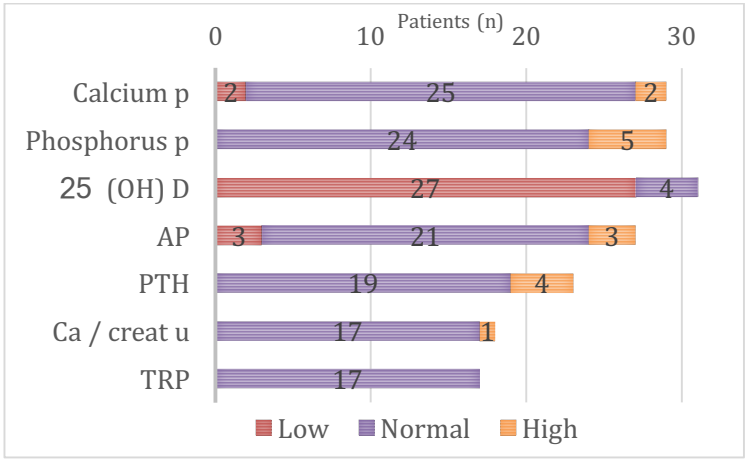


Figure 36. Phosphocalcium metabolism biochemical analysis: classification according to reference values (p=plasmatic; 25 (OH) D = plasma 25-hydroxy-cholecalciferol; AP=alkaline phosphatase; PTH=parathormone; Ca / creat u= urinary calcium / creatinine ratio; TRP=tubular reabsorption of phosphate) (labels correspond to the number of patients)

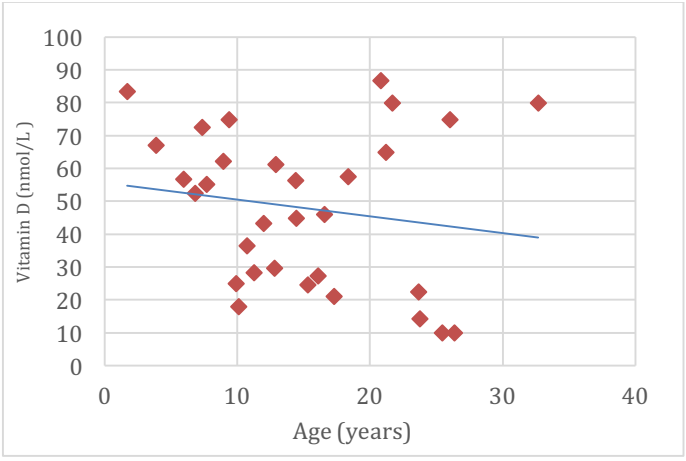


Figure 37. Phosphocalcium metabolism analytical indicators: plasma 25-hydroxy-cholecalciferol (nmol/L), according to age (n=31). A trend line is displayed in blue.

No statistical differences were found concerning nutritional status parameters analysed between the patients group with major central nervous system involvement and that with major skeleton involvement nor between the groups under ERT or not.

VI. QUALITY OF HEALTH

All patients or their guardians (31) answered the quality of health questionnaire (HAQ). Eight patients answered by themselves; the remaining questionnaires were answered by mothers (n=18), fathers (n=2) or other caregivers (n=3).

A. DIFFICULTIES ASSESSMENT

Eating and drinking

The results of the difficulties in eating and drinking are summarized in Table S60.

Globally, the activity that was associated with greater difficulty was "Open a jar or food container by twisting lid" (M=8.7; Mdn=11) and the easiest was "Eat using fingers to pick up food item" (M=3.2; Mdn=0). The activities "Use a knife to butter bread or cut soft foods" and "Pour liquid from carton or jug" were frequently associated with moderately difficulty.

MPS type III patients had higher scores in "eating and drinking" when compared to the other MPS patients group (U=47.5; p=0.023) (Figure 38).

A moderate positive correlation was detected between age and "eating and drinking" score (rho=0.334).

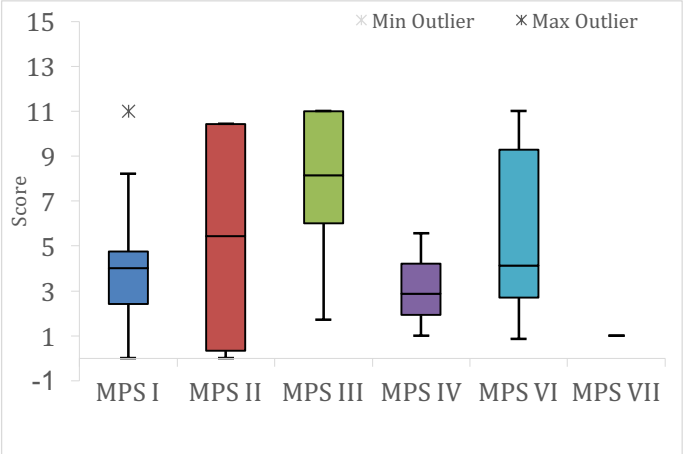


Figure 38. Difficulties assessment – "eating and drinking" domain, per type of MPS (n=31).

Dressing

Answers concerning dressing-related activities are summarized in Table S61.

"Tie shoelaces" was the most difficulty-reported activity. "Put on a front-opening shirt, not including fasteners" was the easiest (mean of difficulty: 7.3 (SD=4.8); median:11 points).

MPS type III patients had higher scores in the "dressing" domain, comparing to the remaining patients group (U=48.0; p=0.040) (Figure 39).

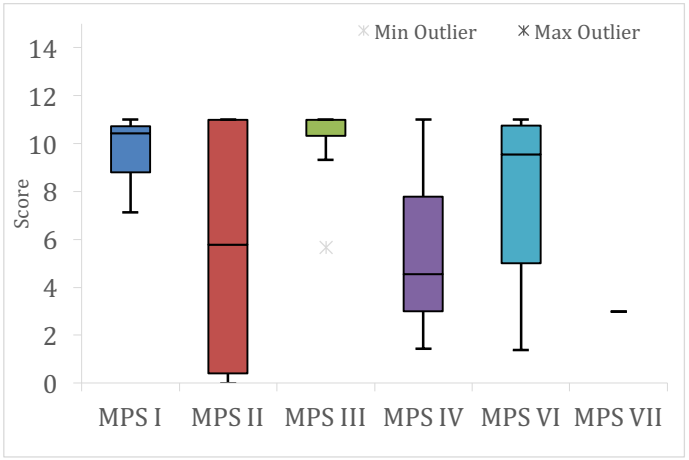


Figure 39. Difficulties assessment – "dressing" domain, per type of MPS (n=31).

Bathing

Results on bathing-related activities are depicted in Table S62.

The activity presenting the highest difficulty among patients was "Cut fingernails with clippers" (M=9.6; SD=3.3; Mdn=11); other activities related to "Wash and dry upper body thoroughly (neck, arms, chest, upper back)", "Wash and dry lower body thoroughly (abdomen, lower back, legs, feet)" and "Brush or comb hair thoroughly (including hair on top of head and back of neck)" presented high difficulty, as well (Mdn= 11).

"Turn tap on and off" was the easiest activity to these individuals (M=5.4; SD=5.3; Mdn=5), followed by "Brush teeth, even if not thoroughly" (M=5.6; SD=5.3; Mdn=4).

Toileting

Answers concerning toileting-related activities are described in Table S63.

"Get on and off toilet without assistance" was the most accessible activity to individuals (M=4.9; SD=5.2; Mdn=1) whereas the hardest was "Wipe self thoroughly after bowel movements" (M=7.2; SD=7.8; Mdn=11).

Still, the activities "Manage clothes before and after toileting" and "Manage toilet seat, get toilet paper and flush toilet" presented median of 10, demonstrating also a high difficulty when performing these.

"Toileting" domain score showed a moderate positive correlation with age of patients (rho=0.328).

Mobility

Results related to mobility are described in Table S64.

"Get in and out of the front seat of a car" was the easiest activity to perform for these individuals (M=5.9; SD=4.5; Mdn=11). The activity with more difficulty associated was "Manage a seat belt or restraint in car" (M=6.7; SD=4.8; Mdn=11).

Two patients (6.5%) used walking aids, namely crutches and cane, for more and for less than half the time of ambulation, respectively. Twelve individuals (38.7%) used a wheelchair for their daily activities, nine in a permanent way. One patient had an electric wheelchair.

A moderate positive, significant correlation between age and HAQ scores on "mobility" was found (rho=0.474; $p<0.01$).

Walking and climbing stairs

Answers related to walking and climbing stairs are summarized in Table S65.

"Walk across level surfaces, such as smooth pavements or driveways" was the less difficult activity (M=5.7; SD=4.8; Mdn=6.5), while the more complex activity was "Walk across rough or uneven surfaces, such as a lawn or a gravel driveway" (M=6.7; SD=4.7; Mdn=10).

HAQ scores on "walking and climbing stairs" domain showed a moderate, positive and significant correlation with age of patients (rho=0.481; $p<0.01$).

Patients with higher scores in "walking and climbing stairs" domain presented lower values of plasma creatinine (rho=-0.510; p<0.05) and araquidonic acid (rho=-0.713; p<0.05) (both strong correlations).

Mean difficulty score reported for all and each domain is presented in Figure 40 and detailed in Table S66.

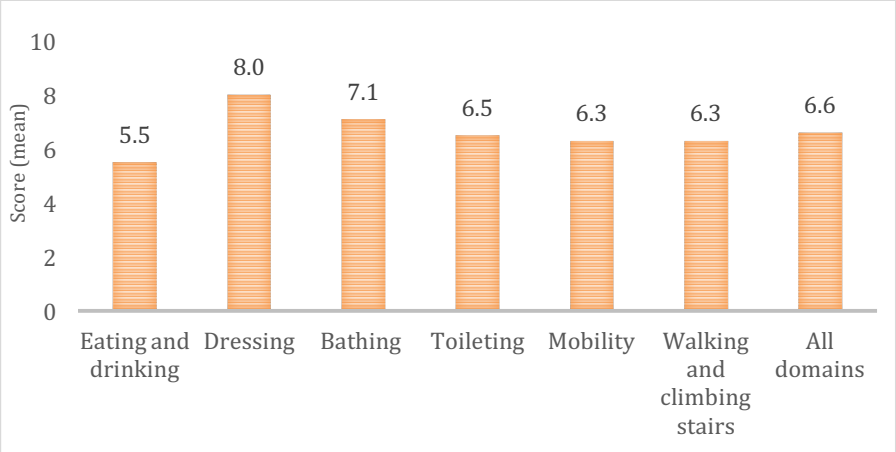


Figure 40. HAQ: mean score obtained in each domain (ranging from 0 "not difficult at all" to 11 points "unable to do") (n=31).

Higher scores in total HAQ score (all domains) were detected in MPS type III patients, compared to the other MPS patients group (U=54.0; p=0.049) (Figure 41). No other significant differences in total HAQ score results were found among MPS type groups.

It was found a moderate positive and significant correlation between age and total HAQ score (rho=0.404; p<0.05).

Six patients (MPS I_5, MPS III_1, MPS III_2, MPS III_7, MPS III_9 and MPS VI_4) were not able to perform (11 points) any of the activities mentioned in the questionnaire.

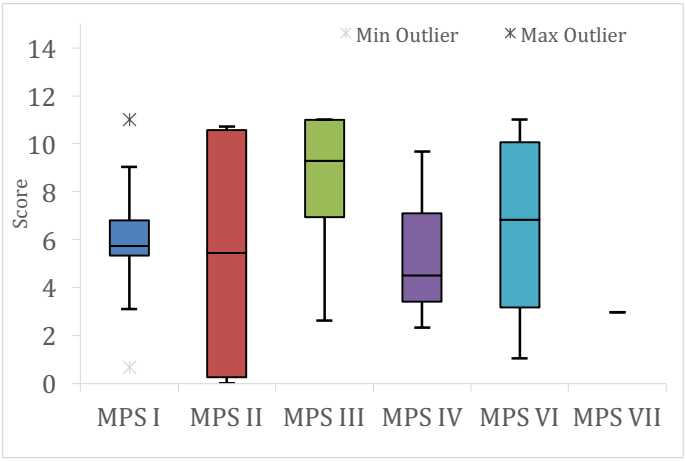


Figure 41. Difficulties assessment – all domains, per type of MPS (n=31).

B. CAREGIVER ASSISTANCE

Results about need for caregiver assistance (independent; minimal, moderate and total assistance) are displayed per domain in Figure 42. Detailed data is displayed in Table S67.

Caregiver assistance total score presented a mean of 3.0 (SD=1.1) and a median of 3, ranging from a minimum of 1 (corresponding to independent) to a maximum of 4 (consistent with total assistance).

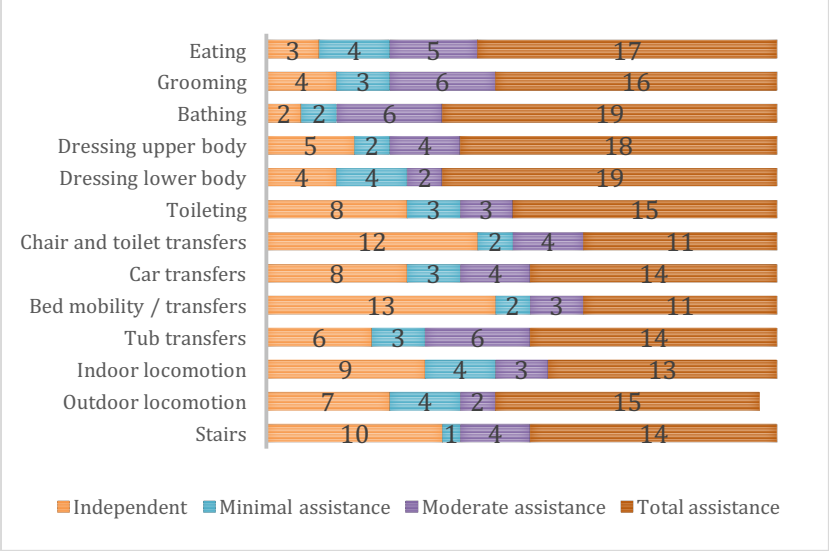


Figure 42. HAQ (caregiver assistance): mode of answers, by domain (n=31).
(labels correspond to the number of patients)

The activity domains with maximum autonomy were “chair and toilet transfers” and “bed mobility / transfers” (“independent” for 41.4% and 44.8% of the patients, respectively). “Bathing” and “dressing lower body” were the domains where dependence was complete (“total assistance” for both in 65.5% of the patients).

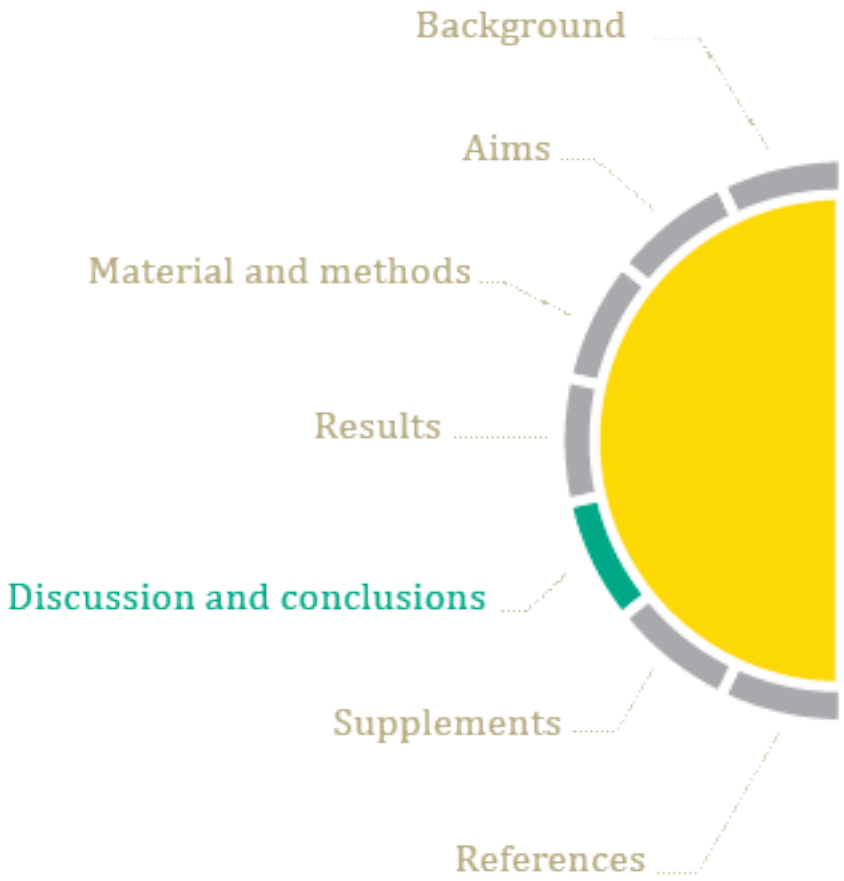
Age and “caregiver assistance” score showed a moderate positive correlation (rho=0.361).

“Caregiver assistance” scores displayed a moderate negative correlation with plasma creatinine (rho=-0.440; $p<0.05$) and araquidonic acid levels (rho=-0.669; $p<0.05$).

No significant statistical correlation was found between HAQ scores and adequacy of nutritional intake.

There was no significant difference in HAQ scores between MPS patients with major central nervous system involvement and those with major skeleton involvement.

No significant difference was found between patients under ERT and others concerning to HAQ scores.


Background
Aims
Material and methods
Results
Discussion and conclusions
Supplements
References

MPS are a protean group of rare, chronic and progressive disorders, associated with severe morbidity and reduced life expectancy, which determine a major impact in quality of health and daily activities. It is widely recognized that chronic degenerative disorders are associated with nutritional status impairment. However, limited research has been done in the field of nutrition of MPS.

This is the first comprehensive study about nutritional status of patients with MPS developed in Portugal. Most of the research conducted in this field relates to enhancing patients' life expectancy and quality, namely through novel treatments. Clinicians have been mostly focused in disease complications and survival. Nutrition has not been a concern until a few years ago [42, 57].

In the last decades, the improvement of care and survival and the diagnosis of less severe phenotypes, due to enhanced awareness of these rare disorders, more patients with a MPS diagnosis are now living until more advanced ages. Since these are degenerative disorders, with several morbidities, other issues, such as nutrition, are currently arising [58, 59].

Laboratorial diagnosis of MPS in Portugal has been done since early 80's in a reference laboratory. In a recent presentation at the 12th International Symposium of the Portuguese Society of Metabolic Diseases (SPDM), held in March 2016, it was reported that MPS diagnosis had been achieved in a total of 159 patients: 37 MPS I, 35 MPS II, 38 MPS III, 19 MPS IV, 23 MPS VI and 7 MPS VII[60].

Clinical care to MPS patients in Portugal is widely distributed in several hospitals. Reference Centres for Inherited Metabolic Diseases have been recently recognized, although Treatment Centres for Lysosomal Disorders have been implemented a few years ago.

The patients included in this study (n=31) were recruited from four different Reference Centres, as a convenience method of sampling.

The above mentioned recruitment method enabled the gathering of the largest possible sample, corresponding to about 20% of the total MPS diagnosis in our country [4]. This could correspond to an even larger parcel of the living MPS portuguese patients, considering the severity of clinical phenotypes in most diagnosed patients and the reduced life expectancy in MPS. Although this is a reduced number for statistical purposes, namely for the analysis of the different MPS types and age groups, it can be considered a rather representative sample of living MPS patients in Portugal.

However, the accomplishment of the study protocol became more difficult due to the recruitment from four different Centres. In fact, it was not completely fulfilled in some of the

centres, because of financial or logistical containments, namely concerning to laboratorial parameters.

Characterization of the sample

The sample was distributed equally through the different age groups considered, except for 0 to 4 years, which included only two male patients. Most of the collaborating clinicians are paediatricians, leading to a prevalence of children and adolescents in our sample. In spite of that, the median follow-up (9.4 years) was long enough for patients to develop the typical clinical phenotypes and complications and allowed to explore the chronic and degenerative characteristics of MPS.

Considering the global estimated prevalence of these diseases, it was expected to see more patients with MPS I, II and III (mainly type B) than MPS VI. This might have been caused by the convenience of recruitment, which included all patients followed at Coimbra, mainly with MPS VI.

MPS II is an x-linked recessive condition, justifying the existence of only male patients in this group of disease [1, 61].
The maximum proportion of adult patients was found in MPS VI group. It might be the cause of a higher degree of impairment and dependence detected in this group.

The absence of patients older than 35 years might be justified by the reduced life expectancy of these patients, who frequently die before reaching adulthood.

Diagnosis of these patients was made early in life in most cases, which may indicate a cluster of severe forms of MPS. This is most relevant for MPS I and MPS VI patients. In MPS I registry published in 2012, there was a consistency of ages at onset and at diagnosis with clinical severity [9]. Diagnosis occurred before 1 year of age in severe, around 4 years in moderate and around 10 years of age in mild phenotypes. In our results, diagnosis of MPS I patients occurred around 2 years of age, consistent with moderate to severe phenotypes.

Still, in this sample some were diagnosed later in life, possibly due to be a less severe form (MPS IV for instance) or to the inability to recognise these diseases some years ago.

A high amplitude of ages at diagnosis was found mainly in MPS II and MPS IV groups, with maximum in patients MPS II_1 and MPS IV_1. These were the only patients where diagnosis was made after 10 years of age.

In a previous study, age at diagnosis and age at onset of symptoms was evaluated in a group of 113 MPS patients [62]. Median age at onset of symptoms was 18 months and diagnosis came about 5 years later, similarly between MPS I, MPS II, MPS IV (subtype A) and MPS VI. In MPS II group developmental delay was established before diagnosis, that happened later than others, around 8 years of age, in median.

As reported in literature, it is common a diagnostic delay of about two to three years after onset of symptoms [63], even in MPS with severe cognitive and somatic disease [61]. Unfortunately, age at clinical presentation was not recorded in the present study, which constitutes one of its limitations.

MPS patients are frequently submitted to chronic pharmacological therapy, due to the numerous associated morbidities. In the recent years, as these patients' life expectancy extended due to new treatments and appropriative palliative care, other morbidities have arisen, as a consequence of the degenerative profile of the disorders [64]. Conditions like epilepsy, musculoskeletal impairment and cardiac dysfunction are becoming more common with advances in age. Anti-epileptic drugs were used by almost a third of our patients. Using these and other chronic medications might cause digestive symptoms and interfere with appetite, impairing the nutritional status of these patients.

Only three patients (less than 10% of the sample) were taking a complete nutritional supplement. These were followed at our centre, where a nutritionist is permanently part of the multidisciplinary team. Vitamin D supplements were frequently used, but probably not in the adequate dosage, taking into consideration the high number of patients with deficiency. It may be hypothesised that these patients might not absorb nutritional supplements correctly or might have higher needs than normal population.

Nutritional intake

Recent nutritional intake was collected, based upon the 24-hour recall questionnaire. Considering that it corresponds to just one day, the representability of usual intake is not warranted. So, for the analysis of adequacy and the correlations with other data, food frequency questionnaire results were used, as these are supposed to be representative of the intake of past year. Indeed, macronutrients distribution respecting to caloric intake was similar between both methods: protein 21.7 vs 20.5%; fat 31.0 vs 32.4; carbohydrates 44.1 vs 48.3% (24-hour recall vs food-frequency questionnaire). This similarity between both methods strengthens the food frequency questionnaire results, as recommended [65].

Food habits were obtained through the food-frequency questionnaire, which reported patients' food intakes by themselves or as understood by caregivers. Even though this type of questionnaire may overestimate nutritional intakes [66] and is only validated for Portuguese adults with no validation when answered by caregivers, it was used, associated with 24-hour recall [67]. Of course, it depends on the caregiver's memory and accuracy, which may also be influenced by their own habits.

A high prevalence of individuals that do not eat healthy food regularly, namely fruits and vegetables, was found. Milk is a daily consumed food, especially reduced-fat milk, as well as yogurts, which are components of the portuguese usual diet. These are also very common in our culture. There is a high intake of sugar-rich beverages, namely iced tea, which is consumed on a weekly basis by most of the patients. These findings bring a lot of concern not only because of the unhealthy pattern in MPS patients but also because this might replicate what is common in our culture. It could be anticipated that a healthy eating pattern would be beneficial in these chronic, progressive diseases preventing and helping to treat some of the associated morbidities. On the other hand, an emotional compensation, common in caregivers of patients with a reduced life expectancy, could contribute to the diet deviation, attempting to attenuate the psychological effects of the diagnosis, with its gloomy prognosis. Another factor that determines food habits is the restriction of specific foods (e.g. milk, due to gastrointestinal disturbances), and its replacement by others. In fact, it was quite frequent in these patients to see a refusal of dairy foods and intake of vegetables substitutes. Some caregivers try to provide a pattern of feeding as healthy as possible (as they think it is), in order to ameliorate or even halt disease progression, by using seeds and grains in high proportions, which in many cases may worsen the gastrointestinal function.

Examining usual nutritional intake, most of the patients showed lower energy, carbohydrates and fiber intake than recommendations. This might be associated with eating difficulties, preferences of inadequate food or even lower appetite. It was not MPS type specific and did not vary with age. Contrary to what might be expected, there was not a statistical correlation between this reduced intake and nutritional status parameters, despite of an apparently high prevalence of patients with analytical signs of malnutrition. Even though, it is recognized that this questionnaire does not include prepared and cooked food, neglecting the amount of fat used (e.g.), for the nutritional intake calculation.

Protein intake was adequate in almost half of the sample, though some still presented lower intakes, and this is more significant in MPS VI patients. Patients aged between 5 to 11 years of

age tend to eat lower protein than recommendations and this might pose a problem in accomplishing growing needs.

Fat intake was adequate in most of the patients. Nevertheless, most of them did not reach recommendations for omega 3 fatty acids. Omega 6 fatty acid intake was also insufficient in most of the individuals.

Age did not seem to bring any influence to eating patterns, what reinforces the need to implement a healthy pattern of eating since early ages. Besides there was not a correlation between level of assistance and adequacy of intake, which means that whether these patients eat by their own or depend on a caregiver, results of adequacy are similar.

Except for vitamin D, vitamin intake was adequate in most of the patients. In fact, almost all patients showed an inadequate intake of vitamin D, even though there was not a correlation between ingestion of this vitamin and its plasma levels. Besides intake, other factors, such as sun exposure, are known to contribute to plasma levels.

Minerals intake was generally adequate, except for manganesium, potassium and calcium. Due to calcium role in bone metabolism, deficient intake in patients in ages with high needs for growth, namely in adolescence, should be corrected. It can be achieved through diet modification or calcium supplementation, if necessary.

Anthropometrics and body composition

In what relates to anthropometric data, significant shifts were detected in height z-score of these group of patients. Except for MPS III group, all MPS types showed a mean shorter stature than references. Height z-score was significantly lower in older patients. These findings are consistent with a generalized, progressive disease with bone involvement. This is quite significant in MPS IV and MPS VI groups, the ones with major skeletal involvement, where height z-score reached values of -10.1 (in the oldest patient) and -8.8, respectively. In fact, dysostosis multiplex is most severe in those MPS types and it is certainly associated with marked short stature.

Analysis of BMI data, using z-score for WHO references, disclosed eutrophy in the majority of the patients. Two presented severe thinness and six were overweighed (one obese). The patients with high BMI z-score were aged mainly between 6 to 10 years of age, except for one 23-year-old MPS VI man (MPS VI_5). Nevertheless, BMI z-score showed a negative correlation with age, crossing z-score 0 in middle of adolescence ages. Indeed, two patients, aged 20 (MPS I_5) and 25 years (MPS III_7), were classified with severe thinness (BMI z-scores of -3.9 and -4.6).

Even though, when this data was compared with specific BMI growth curves (available for MPS II, MPS III and MPS IV), only one patient with MPS III showed overweight. Specific growth curves are not available for MPS VI and MPS VII, so this critical analysis is hindered. Contrary to what would be expected, neither nutritional intake of energy and macronutrients seemed to affect BMI scores, nor differences in BMI were found between MPS types.

Body composition was assessed using a tetrapolar bioelectrical impedance analyser in most of the patients [68]. Minimum body lean mass was found in MPS IV group, one of the types with major skeletal involvement. These results were compared with data published in healthy individuals, enabling the identification of three patients with overfat and three with extremely low fat-free mass index. Two of these were classified with severe thinness, one using BMI z-score and the other with specific curves for disease. These results are arguable, since this method, as well as the predictive equations used, may not be adequate to estimate body composition in these patients. MPS is a group of diseases with phenotypical characteristics, including somatic morphological modifications, which bring to discussion the possible body asymmetry. This might impair the validity of this method as an evaluation tool in MPS patients, though it can be used in the longitudinal follow up. Still, it was the only technique available. Phase angle was also acquired and considering it is a parameter obtained almost directly, without the need of predictive equations, results were reviewed and used in statistical analysis.

In this sample, most of the patients (13 in 19) showed lower phase angle than published references, when stratified for BMI. Indeed, most of the results are lower than 5.0°, value frequently associated with bad prognosis in critical status of chronic disease patients. Phase angle was not assessed in MPS I and MPS VII individuals. MPS IV group was the only one where all the patients were found to have low angles. The three patients mentioned before, who presented extremely low fat-free mass index (MPS II_4; MPS III_7; MPS III_9) also showed low phase angle. All of them displayed low plasma creatinine; one (MPS II_4) also showed low pre-albumin and normal RBP. In the other two (MPS III_7; MPS III_9) pre-albumin and RBP were not measured.

In line with other publications, phase angle results showed a positive moderate correlation with age and with lean body mass. Nevertheless, nutrient intake was not related to phase angle results. So, low phase angle values found in this study population may be a consequence of the severity of disease more than just an indicator of a poor nutritional status associated with inadequate intake.

A possible link with low phase angle values found in our sample is the existence of an inflammatory status, common in MPS patients [69, 70]. In this study c-reactive protein was

normal in the majority of the patients, though the c-reactive protein assay used was not the high sensitivity one [71]. It would have been interesting to have tested for other inflammation markers such as tumor necrosis factor – alpha, interleukin-1 or interleukin-6 in these patients [70, 72].

Resistance test

A resistance walking test was performed aiming to relate functional abilities to nutritional status. MPS II patients were the group with higher results in 6-MWT.

Most of the patients walking a shorter distance (<25th centile), are older than 12 years of age, showing a tendency in functional skills deterioration associated with age. This is in accordance to the chronic and degenerative characteristic of MPS, although it was not a statistically significant correlation.

Twelve patients were unable to walk, due to their severe clinical status. Eight are completely dependent on wheel chair. All of these were older than 20 years, except for one 10-year-old patient, which corroborates the progressive severity of this disease with age.

Laboratorial data

Some laboratorial parameters were analysed with the purpose of better characterization of the patients' nutritional status. Although there was a pre-defined protocol, some of the parameters were not analysed in all cases. Some of them, more specific and not routinely performed in the hospital laboratories, dependent of administrative authorization to be done in external laboratories, such as essential fatty acid profile, were sometimes missed. Laboratorial parameters used regularly for assessment of patients were generally requested, although with a few exceptions.

The lack of full accomplishment of the pre-defined laboratorial protocol is a limitation of this study, disabling some of the potential findings. Besides this, the analysis were performed in diverse laboratories, raising the discussion of uniformity of procedures, analytic methods and reference values.

Total protein, albumin and transferrin plasma levels were normal in most of individuals. Two individuals showed moderate hypoalbuminemia. One of them (MPS III_2) presented the highest values of ALT and AST and the lowest phase angle result, without any significant modification of body composition. The other (MPS I_5) showed severe thinness as well as low levels of plasma pre-albumin and RBP, even though she was the one with the highest recent caloric intake and

was taking a complete nutritional supplement regularly. Unfortunately, this patient did not perform body composition assessment. Nevertheless, plasma albumin is not a reliable parameter to nutritional status assessment, as it can be influenced by numerous factors, namely liver dysfunction and renal losses [73].

Most of the patients who performed pre-albumin and RBP analysis showed low levels. As these two proteins are reliable markers of nutritional status, this may indicate the presence of malnutrition status in most of them. However, other factors may contribute to the low levels, namely the presence of an inflammatory status, which has been described in MPS patients. As stated before, it was not adequately evaluated in this sample, since C-reactive protein was the only parameter studied. Phase angle and plasma fatty acid results corroborate the malnutrition status in most of the study population.

It is well known that pre-albumin and RBP are highly sensitive to protein intake [74]. However, a significant correlation between these laboratorial parameters and recent or usual protein intake was not present in this sample, which may be due to its limited number.

Age did not seem to influence plasma pre-albumin and retinol-binding protein levels. Though, a tendency line to lower values in older patients was identified in these parameters, not in agreement with published data for healthy subjects [75].

Concerning to lipid profile, total cholesterol, LDL-cholesterol and triglycerides were normal in most of the patients. The three patients who showed higher LDL-cholesterol values than references (MPS III_7; MPS IV_1; MPS VI_3), presented normal HDL-cholesterol levels. Interestingly, HDL-cholesterol was low in the majority of analysed patients, which may be associated with poor nutritional status or an inflammatory and oxidative phenotype [76, 77]. Low HDL-cholesterol levels are a risk factor for atherosclerosis, which has not been a problem in MPS patients, probably due to the reduced life span. However, it would be interesting to analyse the lipid profile in a larger cohort in order to conclude if low HDL-cholesterol is a consistent finding in MPS.

Concerning to plasma essential fatty acids, the majority of patients showed normal values of araquidonic, docosahexaenoic and eicosapentaenoic acid levels.

Still, older patients showed significantly higher levels of araquidonic and docosahexaenoic acids. The ratio araquidonic acid : (docosahexaenoic acid + eicosapentaenoic acid) tends to be higher in older patients and, even without statistical significance, in patients with worst performances in 6-MWT and in those with higher dependence scores in HAQ. In a recently published study, this fatty acid pattern was associated with lower physical performances [78],

which is in line with these results. Nevertheless, the role of these fatty acids in inflammatory status and immune response is well known [79, 80].

No correlation was found between plasma essential fatty acids levels and nutritional intake. However, this analysis was only performed in a small part (38.7%) of the study sample, which impaired the adequate statistical analysis.

Most patients showed normal plasma values of vitamins, except for vitamin D. Only two of eleven patients with high plasma levels of B12, were taking a nutritional supplement. These were the ones with the highest values. High levels of this vitamin have been associated with cancer and considered a mortality risk factor in patients with a few severe diseases [81]. However, the mechanisms underlying this association are not yet elucidated, and some controversial data has been published [82-84]. To our knowledge, MPS patients are not prone to cancer development. On the other hand, only the long-term follow-up of those patients with high vitamin B12 levels might confirm the link with higher mortality.

Low levels of vitamin A (6 in 23 patients) or vitamin E (6 in 22 patients) were found. Since those were different patients, it corresponds to more than half (12) of the analysed sample. Apparently, the levels of these vitamins were not related with the nutritional intake. The low levels of vitamins A and E may be an indirect evidence of oxygen free radicals damage, possibly correlated with a potential inflammatory status associated to MPS. This fact, as well as negative correlation of vitamin E levels with age, would support the use of anti-oxidants supplements as a co-adjuvant therapy in these patients [69, 70, 72, 85-90]. In this context, evaluation of vitamin C status would have been interesting.

Plasma concentrations of sodium, potassium, magnesium, selenium and zinc were normal in the majority of patients. The only two patients with high values of magnesium were taking a nutritional supplement.

The great proportion of analysed patients showed normal liver and renal parameters, as well as total blood count analysis, as anticipated in MPS [1].

Creatinine levels are associated with muscle mass and glomerular renal function and, as expected, did not show any statistical correlation with nutritional intake. The majority of the 31 patients (77.4%) showed low levels of plasma creatinine. This might be due to poor muscle bulk in these patients. Contrary to what was predictable in the sample age range, a weak negative correlation (although with no statistical significance) was found between age and creatinine levels. Also, patients with higher scores in “walking and climbing stairs” domain

presented lower values of plasma creatinine (strong correlations). It would be important to understand if those are independent or related factors in those correlations.

One of the most characteristic involvements in MPS is bone dysplasia, which determines the low stature in many patients, mostly those with MPS IV and MPS VI types. Low bone mineral density and muscle and skeletal pain have been reported in MPS [59, 91-94]. Although a few patients had chronic pain and in some, bone mineral density evaluation had been done, its analysis was not one of the objectives of the present study.

In this sample, phosphocalcium metabolism parameters were globally normal, except for high PTH (4 in 23 patients) and vitamin D (27 in 31 patients) levels. Vitamin D deficiency is rather common in general population and also in MPS patients [59, 93, 95, 96]. Low vitamin D values were present in 87.1% of the patients and deficiency status was detected in about half of the analysed sample. Three of the four patients with normal vitamin D values were taking a supplement of this vitamin.

Considering that MPS is a degenerative disease and consequently patients are frequently dependent on caregiver to have sun exposure, vitamin D status should be regularly monitored and supplemented as needed [59].

Quality of health

Quality of health questionnaires are important tools for the evaluation of disease impact in daily activities and need for caregiver assistance in chronical diseases [47]. Unexpectedly, no correlation was found between quality of health and nutritional intake.

In our sample, a significant number of patients showed high levels of difficulty for several daily tasks associated with high levels of caregiver dependence, as expected. Six in 31 patients (19.4%) were not able to perform any of the questioned activities. As could be anticipated, older patients presented higher significant levels of difficulty in most tasks ("walking and climbing stairs"; "mobility"; "toileting"; "eating and drinking"), in total HAQ score and need of caregiver assistance [47, 97].

Patients with higher scores in "walking and climbing stairs" domain and "caregiver assistance" presented significant lower values of plasma creatinine and araquidonic acid (strong correlations), which could be associated to muscle wasting and / or inflammation.

Nutrition across MPS types

MPS are a heterogeneous group of multisystem diseases, which differ on major organ involvement. Age at presentation and survival are also variable, not only among MPS types, but also between patients of each type. ERT, which began in 2003 for MPS I, is now available for most MPS types [1, 98]. Despite current intense clinical investigation in this field, which includes gene therapy for some MPS types, the efficacy of ERT, as well as other available treatments, like stem cell transplantation, is far from optimal [99].

With the attempt to identify possible differences in nutritional status, nutritional intake and quality of health between MPS types, the corresponding parameters were analysed in more detail. MPS VII was excluded from this analysis, due to the existence of only one patient.

Despite being expected that age at diagnosis is more dependent on disease severity and age of presentation of first symptoms (as well as clinical skill) than on the MPS type, in a previously study in a group of 113 MPS patients, diagnosis happened in similar ages in MPS I, MPS IV and MPS VI groups and later in MPS II patients [62]. In the present study, age at diagnosis was similar across the several MPS types, though in MPS I and MPS VI group, lower ages were referred, without statistical significance. This may be associated with the presence of more severe forms of the disease in these groups, as expected [9]. Other factors, like less awareness about these disorders and difficulties in performing the laboratorial diagnosis in the past may be relevant in this issue.

MPS patients have, characteristically, growth impairment compared to healthy subjects. Across the different types, some (distinctly MPS IV and VI) are distinguished by more severe skeletal involvement and a shorter final stature. In this study, except for MPS III group, all types of MPS showed a mean stature shorter than WHO references, worsening with age, as anticipated due to the progressive feature of the diseases. This was noteworthy in MPS IV and MPS VI groups, the ones with major skeletal involvement as previously referred, where height z-score reaches values of -10.1 (in the oldest patient) and -8.8, respectively, in agreement to other studies [58]. On the other hand, weight and BMI were similar along the different MPS types.

Body composition results were analogous across the different MPS types, with minimum body lean mass in the MPS IV group, although this difference was not statistically significant.

Phase angle was not assessed in MPS I and MPS VII patients. Low values were predominant in the entire analysed sample. No significant differences in phase angle were found between MPS types. However, all MPS IV patients showed low values, possibly conditioned by their reduced stature, which has been described as a predictive factor for phase angle [100].

Other parameters might sustain these results about nutritional status assessment. Some laboratorial results were compared through the different MPS types, as mentioned in the methods section.

The group of MPS VI patients presented significantly lower values of plasma RBP and vitamins A and E, as well as higher araquidonic acid levels than the others. Although there was not a significant difference, most of the patients showing lower results of plasma magnesium were MPS VI patients.

In accordance with these results, many MPS patients, mostly in MPS VI individuals, may have a subclinical status of undernutrition and/or a chronic inflammatory situation [5].

Although energy intake is inadequate in most of the patients in analysis, there were no significant difference between the several MPS types. Besides this, protein intake was inadequate in almost half of the sample and this is significant among MPS VI patients, but not significantly different from the other MPS groups. Also, plasma levels of vitamin E were higher in MPS IV patients, which does not corroborate the previously formulated hypothesis.

Pre-albumin, RBP and vitamin A levels were significantly higher in MPS III patients compared to the others. Indeed, MPS III as a group is associated with less somatic disease severity, as expected [5].

MPS III patients are mostly affected in cognitive and behavioural areas. This may lead to higher levels of assistance needed[5], despite the less severe somatic involvement, as it was demonstrated in our cohort. Notwithstanding the high levels of difficulties detected in all patients, MPS III individuals stand out with the highest scores in HAQ, when comparing with the other MPS groups. This is more significant in the domains associated with eating and grooming, as expected.

Other parameter that might be related with disease type is the performance on 6-MWT. In this resistance test, MPS II patients showed the highest results, demonstrating the highest capability in walking long distances. MPS III patients were expected to walk longer distances, due to less physical impairment, but in most of them the results became lower due to difficulties in understanding the command given.

As a general rule, MPS types IV and VI present major skeletal impairment with relative preservation of intellectual functions, whereas MPS I, II, III and VII are more frequently associated with cognitive injury[5]. We have analysed the differences in nutritional intake, and nutritional status, as well as in quality of health between these two clusters of patients.

Patients with predominant skeletal affection frequently have pain and mobility impairment. These are expected to cause high levels of dependency on the caregiver due to greater difficulties in performing daily activities, as well as less functional abilities, namely in mobility domains, like walking and climbing stairs[47]. Besides this, the immobility may also contribute to abnormalities in some of the nutritional status parameters analysed, like phase angle, lean body mass, plasma proteins and vitamin D.

On the other hand, patients with predominant cognitive impairment could present higher levels of need for caregiver assistance [47]. This might result in better food habits, as the dependence of the caregivers may end in better choices, and consequently, nutritional intake might be more adequate.

Notwithstanding with those expectations, results from nutritional intake and nutritional status analyses were similar between the above mentioned MPS patient groups. Similarly, no differences were found between both groups, relating to quality of health and resistance test parameters.

ERT is available for MPS I, MPS II, MPS IV and MPS VI patients [1]. It would be expected that patients submitted to ERT would have better quality of health and nutritional status than untreated patients with the same MPS type, corroborating the evidence of benefits of this kind of therapy. To our knowledge, no studies seeking for that evidence have yet been done, perhaps because these are novel therapies and not all the patients with these rare diseases are still submitted to this treatment. On the other hand, large cohorts, needed for that investigation, are difficult to assembly.

In this sample, patients treated with ERT showed lower levels of pre-albumin and vitamin A than those not under ERT. No other differences relating to nutritional intake and quality of health were found between the two groups. It is questionable to analyse the effects of ERT comparing patients with diverse MPS types. We did so, since our sample was small for any results with statistical significance. However, since the no-ERT group included all MPS III patients and only a few from the other MPS types the lower levels of pre-albumin and vitamin A might be explained by the somatic severity of the patients submitted to ERT.

Conclusion

In this study, a comprehensive assessment of nutritional status of 31 MPS patients was performed and related to nutritional intake and quality of health.

Anthropometric data was adequate to growth references for these diseases and body composition (fat and lean mass) showed, in general, adequate results. However, other nutritional status indicators like phase angle, pre-albumin, RBP, creatinine, HDL-cholesterol, vitamin A, D and E and essential fatty acids were lower than references in a significant number of patients.

Nutritional intake was considered adequate in most of the patients, except for energy, carbohydrate and fiber intake, which was low in the bulk. The majority of the patients had inadequate food habits, which can enhance disease progression.

As expected, this study cohort showed high levels of difficulties and, consequently low levels of autonomy.

Some findings (difficulty and need of caregiver assistance and low BMI z-score, e.g.) were more prominent in older ages, as could be anticipated in degenerative diseases.

As widely mentioned in the literature, MPS patients should be regularly monitored by a multidisciplinary team, which must include nutritionists, specialized in inborn errors of metabolism, with know-how in degenerative diseases [101-103].

MPS patients should be subjected to anthropometric surveillance using specific growth curves, as well as body composition assessment and screened for abnormalities in specific parameters like pre-albumin and vitamin D, at least annually. If not avoided, deficits should be corrected, as earlier as possible, in order to optimize the patients' condition.

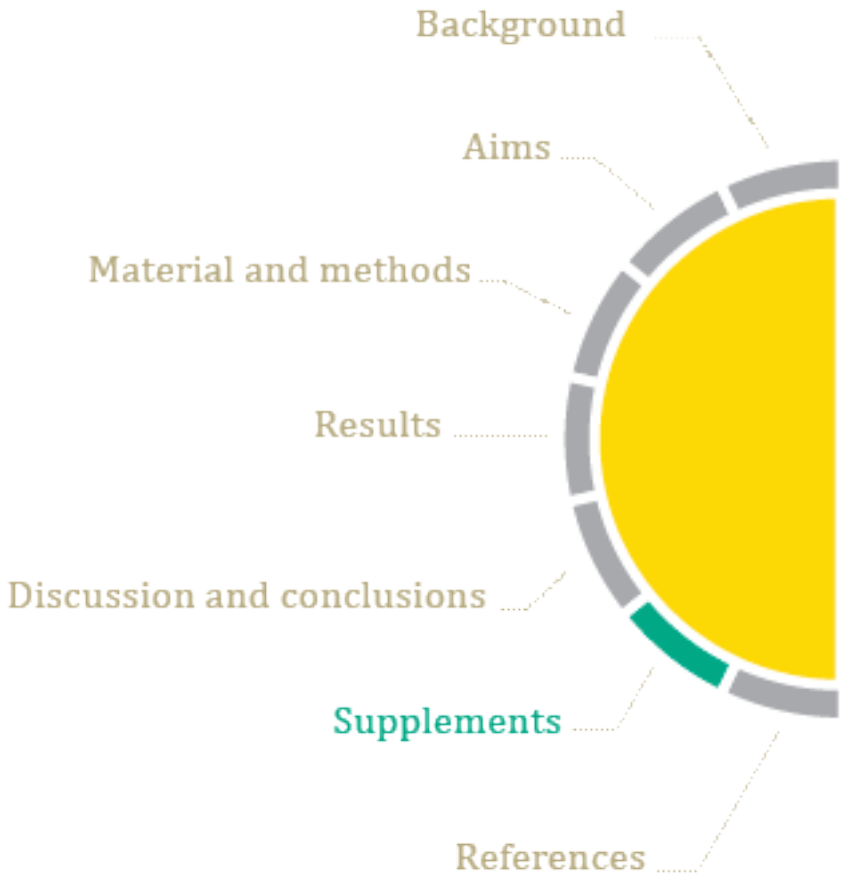
Aiming to provide a better clinical surveillance in order to improve quality of health of MPS patients, through prevention of nutritional deficits and other treatable complications, a nutrition follow-up protocol proposal follows.

Assessment	Methods / indicators	At diagnosis	Follow-up frequency	Pre-ERT
Food and nutritional intake	24-hour recall (representative day) Nutritional intake: compare with recommendations[a]	✓	Every visit	✓
Growth[b]	Weight Height / length Body mass index Head circumference (≤ 36 months)	✓	Every visit	✓
Body composition[c]	Lean body mass Fat body mass Total body water Phase angle	✓	Annual *(more often if nutritional therapy is implemented)*	✓
	Bone mineral density[d]	-	Annual or every other year	-
Nutritional blood biomarkers[e]	Creatinine Pre-albumin Retinol binding protein Vitamin A, D and E Vitamin C Calcium, phosphorus Magnesium Selenium Zinc Alkaline-phosphatase (bone specific, if available) Calcitonin Parathormone	✓	Annual	✓
Inflammatory markers[e]	Tumour necrosis factor – **α** Interleukin-6	✓	Annual	✓

Table 4. Nutritional follow-up protocol – a proposal for MPS patients. [a]Dietary reference intakes; [b]See growth curves appropriated to each type of MPS; [c]Bioelectrical impedance or dual-energy X-ray absorptiometry; [d] from 6 years of age plasma analysis; [e] to screen for inflammation

Finding	Measure
Inadequate food habits	Encourage healthy food intake
Insufficiency / deficiency of vitamin D	Supplement with cholecalciferol or similar Encourage sun exposure
Other vitamin or mineral deficiency	Consider correction
Malnutrition / wasting suspicion	Optimize nutritional intake; consider nutritional supplements Encourage physical activity
Inflammatory status	Consider anti-oxidant therapy

Table 5. Nutritional follow-up protocol – major findings and measures


Background
Aims
Material and methods
Results
Discussion and conclusions
Supplements
References

Patient ID	Type of MPS	Gender	Current age*	Age at diagnosis	ERT (yes / no)	Pharmacological treatment	Nutritional supplements
MPS I_1	MPS I	Male	1.7	0.8	Yes	-	
MPS I_2		Female	6.8	0.3	Yes	GE, OT	Vigantol®, Folicil®, Magnesium B®
MPS I_3		Female	15.3	2.0	Yes	D	
MPS I_4		Male	3.9	2.0	No	GE, ACEI, OT	Folicil®, Magnesona®
MPS I_5		Female	20.8	6.0	Yes	AE, D, ACEI, OT	Fortini powder®, Vigantol®
MPS II_1	MPS II	Male	16.6	12.3	Yes	-	Vigantol®
MPS II_2		Male	9,0	2.6	Yes	AE, AP, OT	
MPS II_3		Male	18.4	-	Yes	ACEI	
MPS II_4		Male	11.3	2.3	Yes	AP, GE, OT	
MPS III_1	MPS III	Female	23.8	3.0	No	AE, HP	Centrum®
MPS III_2		Female	10.8	2.5	No	AE, AP	
MPS III_3		Male	10.1	3.5	No	AP	
MPS III_4		Female	9.4	7.9	No	MF	
MPS III_5		Female	12.9	1.5	No	AP, HP	
MPS III_6		Male	12.8	7.0	No	MF, AP	
MPS III_7		Male	25.4	1.1	No	AE	Fresubin protein®
MPS III_8		Male	6.0	2.0	No	AP	
MPS III_9		Male	26.3	2.3	No	AE, D, ACEI	
MPS IV_1	MPS IV	Female	16.1	11.0	Yes	HP, GE, OT	
MPS IV_2		Female	12.0	5.0	Yes	-	
MPS IV_3		Female	32.7	0.5	No	AN, D	R73®, Cebiolon®
MPS VI_1	MPS VI	Male	7.7	1.0	Yes	-	
MPS VI_2		Female	17.3	3.0	Yes	-	
MPS VI_3		Male	14.4	5.0	Yes	D, ACEI	
MPS VI_4		Female	26.0	-	Yes	AE	Vigantol®
MPS VI_5		Male	23.7	3.0	Yes	AE, ACEI	
MPS VI_6		Male	21.7	1.1	Yes	AE, AN, OT	Vigantol®
MPS VI_7		Female	21.2	2.0	Yes	ACEI	Decalcit®, Ferrum haussman®
MPS VI_8		Female	7.4	2.0	Yes	ACEI, OT	
MPS VI_9		Male	14.4	1.3	Yes	ACEI	
MPS VII_1	MPS VII	Male	9.9	0.1	No	AE	

Table S1. Characterization of sample: general description per individual [age at assessment; AE=antiepileptic drugs, MF=methylphenidate; AP=antipsychotic drugs; HP=hypnotic drugs (melatonine, Halcion®, antiH1); AN=analgesic drugs; GE=gastrointestinal drugs; D=diuretics; ACEI=angiotensin converting enzyme inhibitor(ACE inhibitors); OT=other]*

Age group	Male	Female	Total
0-4 years	2 (11.8%)	0 (0.0%)	2
5-11 years	6 (35.3%)	5 (35.7%)	11
12-18 years	5 (29.4%)	4 (28.6%)	9
>18 years	4 (23.5%)	5 (35.7%)	9
Total	17 (54.8%)	14 (45.2%)	31

Table S2. Characterization of sample: distribution per gender and age group.

Age group	n	Mean	SD	Median	Minimum	Maximum
MPS I	5	2.2	2.3	2.0	0.3	6.0
MPS II	3	5.7	5.7	2.6	2.3	12.3
MPS III	9	3.4	2.4	2.5	1.1	7.9
MPS IV	3	5.5	5.3	5.0	0.5	11.0
MPS VI	8	2.3	1.3	2.0	1.0	5.0
MPS VII	1	0.1	.	0.1	0.1	0.1
Total	29	3.2	3.0	2.3	0.1	12.3

Table S3. Characterization of sample: age at diagnosis (in years), and type of MPS (n=29).

Nutrient	Mean	SD	Median	Minimum	Maximum
Energy (Kcal)	1762.2	523.3	1824.3	840.3	2577.8
Energy (Kcal/Kg/day)	*65.4*	*29.5*	*52.5*	*20.2*	*133.5*
Protein (g)	93.0	28.5	98.3	31.4	136.1
Protein (% / energy)	*21.7*	*6.3*	*21.1*	*11.2*	*37.8*
Fat (g)	60.3	22.1	60.2	20.3	115.6
Fat (% / energy)	*31.0*	*6.9*	*30.8*	*17.4*	*43.4*
Saturated fatty acids (g)	17.5	8.2	15.7	6.0	44.7
Saturated fatty acids (%)	*8.9*	*2.6*	*8.4*	*5.1*	*15.7*
Monounsaturated fatty acids (g)	24.3	9.3	24.4	8.3	41.9
Monounsaturated fatty acids (%)	*12.6*	*3.8*	*12.5*	*5.1*	*22.7*
Polyunsaturated fatty acids (g)	9.0	4.8	7.7	3.9	21.9
Polyunsaturated fatty acids (%)	*4.6*	*2.0*	*4.0*	*1.9*	*9.7*
Trans fatty acids (g)	0.6	0.4	0.5	0.0	1.6
Cholesterol (mg)	271.3	173.6	259.4	44.8	911.7
Total carbohydrates (g)	210.8	83.1	208.5	61.2	395.3
Total carbohydrates (%)	*44.1*	*9.0*	*42.5*	*21.7*	*66.1*
Mono + disaccharides (g)	94.0	54.3	85.3	13.5	259.9
Fiber (g)	14.9	8.3	14.1	1.9	42.9

Table S4. Recent nutritional intake of 31 patients: energy and macronutrients.

Nutrient	Mean	SD	Median	Minimum	Maximum
Vitamin A (µg)	632.4	464.0	510.6	0.7	1732.8
Carotene (µg)	2380.6	2335.9	1872.0	27.5	9711.5
Vitamin D (µg)	7.4	7.6	4.4	0.3	34.0
Vitamin B_6 (mg)	2.1	1.6	1.9	0.5	9.8
Vitamin B_{12} (µg)	2.7	1.2	2.7	0.5	5.5
Vitamin C (mg)	110.2	98.8	79.3	.0	361.6
Sodium (g)	2.8	1.0	2.8	1.0	6.3
Potassium (g)	2.9	1.2	2.9	1.1	5.6
Calcium (mg)	817.8	493.3	710.2	58.6	2484.8
Phosphorus (mg)	1113.9	433.0	1041.0	384.7	2150.0
Magnesium (mg)	213.8	80.6	203.9	79.9	383.6
Iron (mg)	12.9	17.4	9.6	2.7	101.8
Zinc (mg)	9.3	4.1	8.9	2.7	19.1

Table S5. Recent nutritional intake of 31 patients: micronutrients.

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**	**Never / less than once a month**	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**
Whole milk (250ml)	**22**	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0
	(88.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Reduced-fat milk (250ml)	3	1	1	3	2	**9**	5	0	1	0	0	0	0	0	0	0	0	0
	(12.0%)	(4.0%)	(4.0%)	(12.0%)	(8.0%)	**(36.0%)**	(20.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Skimmed milk (250ml)	**23**	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	(92.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Yogurt (one with 125g)	8	0	0	4	1	**9**	3	0	0	0	0	0	0	0	0	0	0	0
	(32.0%)	(0.0%)	(0.0%)	(16.0%)	(4.0%)	**(36.0%)**	(12.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Cheese (serving 30g)	**14**	2	3	4	0	1	1	0	0	0	0	0	0	0	0	0	0	0
	(56.0%)	(8.0%)	(12.0%)	(16.0%)	(0.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Dairy desserts (one or a dessert plate)	**14**	2	7	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	(56.0%)	(8.0%)	(28.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Ice-cream (one or two servings)	**13**	1	0	1	0	0	0	0	0	3	0	4	1	2	0	0	0	0
	(52.0%)	(4.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(12.0%)	(0.0%)	(16.0%)	(4.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

Table S6. Usual nutritional intake: distribution of frequency, annual and seasonal of dairy products.

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	1 - 3 / month	1 / week	2 - 4 / week	5 - 6 / week	1 / day	2 - 3 / day	4 - 5 / day	≥ 6 / day	Never / less than once a month	1 - 3 / month	1 / week	2 - 4 / week	5 - 6 / week	1 / day	2 - 3 / day	4 - 5 / day	≥ 6 / day
Eggs (one)	9	**10**	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(36.0%)	**(40.0%)**	(16.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Chicken (two pieces or 1/4 chicken)	5	2	7	**9**	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	(20.0%)	(8.0%)	(28.0%)	**(36.0%)**	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Turkey, rabbit (one portion or two pieces)	**8**	3	6	**8**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(32.0%)	(12.0%)	(24.0%)	**(32.0%)**	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Cow, pork and goatling meats (120g)	3	3	**8**	7	2	1	0	0	0	0	0	1	0	0	0	0	0	0
	(12.0%)	(12.0%)	**(32.0%)**	(28.0%)	(8.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Liver (cow, pork or chicken) (120g)	**17**	3	1	2	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	(68.0%)	(12.0%)	(4.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Viscera or entrails (100g)	**22**	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	(88.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Ham and sausage (two slices or three slices)	**10**	1	3	8	1	2	0	0	0	0	0	0	0	0	0	0	0	0
	(40.0%)	(4.0%)	(12.0%)	(32.0%)	(4.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Sausages (3 medium)	5	5	**9**	5	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	(20.0%)	(20.0%)	**(36.0%)**	(20.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Bacon (two slices)	**24**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(96.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

Table S7. Usual nutritional intake: distribution of frequency, annual and seasonal of egg, meat and derivatives.

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**	**Never / less than once a month**	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**
Fatty fish such as sardines, mackerel, horse mackerel, salmon, etc. (a portion of 125g)	4 (16.0%)	6 (24.0%)	5 (20.0%)	**9 (36.0%)**	0 (0.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Lean fish such as hake, pouting, gold, etc. (a portion of 125g)	4 (16.0%)	4 (16.0%)	7 (28.0%)	**8 (32.0%)**	2 (8.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Cod (an average set)	7 (28.0%)	6 (24.0%)	**9 (36.0%)**	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Canned like tuna, sardines, etc. (a can)	**11 (44.0%)**	7 (28.0%)	2 (8.0%)	5 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Squid and octopus (a portion of 100g)	**17 (68.0%)**	5 (20.0%)	2 (8.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Shrimp, clams, mussels, etc. (a dessert plate)	**20 (80.0%)**	2 (8.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table S8. Usual nutritional intake: distribution of frequency, annual and seasonal of fish and other sea products.

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**	**Never / less than once a month**	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**
olive oil (one tablespoon)	6	3	**7**	4	1	4	0	0	0	0	0	0	0	0	0	0	0	0
	(24.0%)	(12.0%)	**(28.0%)**	(16.0%)	(4.0%)	(16.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
sunflower oils, corn or soybean (a tablespoon)	**17**	4	1	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0
	(68.0%)	(16.0%)	(4.0%)	(0.0%)	(0.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
margarine (one teaspoon)	**13**	1	5	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	(52.0%)	(4.0%)	(20.0%)	(20.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
butter (one teaspoon)	**9**	0	4	4	3	4	0	0	0	0	0	1	0	0	0	0	0	0
	(36.0%)	(0.0%)	(16.0%)	(16.0%)	(12.0%)	(16.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

Table S9. Usual nutritional intake: distribution of frequency, annual and seasonal of oils and other fats

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	1 - 3 / month	1 / week	2 - 4 / week	5 - 6 / week	1 / day	2 - 3 / day	4 - 5 / day	≥ 6 / day	Never / less than once a month	1 - 3 / month	1 / week	2 - 4 / week	5 - 6 / week	1 / day	2 - 3 / day	4 - 5 / day	≥ 6 / day
White bread or toast (one or two sandwiches)	4 (16.0%)	1 (4.0%)	1 (4.0%)	**8 (32.0%)**	2 (8.0%)	6 (24.0%)	3 (12.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Bread (or toast) full, rye or mixture (one or two sandwiches)	**15 (60.0%)**	2 (8.0%)	1 (4.0%)	2 (8.0%)	1 (4.0%)	2 (8.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Corn bread or corn bread of Avintes (a slice of 80g)	**20 (80.0%)**	2 (8.0%)	1 (4.0%)	2 (8.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Muesli cereal flakes, corn flakes, etc. Chocapic (a cup without milk)	**10 (40.0%)**	2 (8.0%)	4 (16.0%)	3 (12.0%)	1 (4.0%)	4 (16.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Rice (middle plate)	3 (12.0%)	0 (0.0%)	1 (4.0%)	**14 (56.0%)**	5 (20.0%)	1 (4.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Pasta like spaghetti, macaroni, etc. (half plate)	4 (16.0%)	0 (0.0%)	2 (8.0%)	**14 (56.0%)**	4 (16.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Home fries (half plate)	**11 (44.0%)**	5 (20.0%)	3 (12.0%)	5 (20.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Tortilla chips (small package)	**13 (52.0%)**	6 (24.0%)	2 (8.0%)	2 (8.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Boiled potatoes, baked, stewed and pureed (two medium potatoes)	1 (4.0%)	1 (4.0%)	5 (20.0%)	**15 (60.0%)**	2 (8.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table S10. Usual nutritional intake: distribution of frequency, annual and seasonal of bread, cereals and similar.

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**	**Never / less than once a month**	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**
Maria type crackers, water and salt or full (three wafers)	5	2	**10**	2	1	1	3	0	0	0	1	0	0	0	0	0	0	0
	(20.0%)	(8.0%)	**(40.0%)**	(8.0%)	(4.0%)	(4.0%)	(12.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Other crackers or biscuits (three wafers)	**12**	3	5	2	0	1	1	0	0	0	1	0	0	0	0	0	0	0
	(48.0%)	(12.0%)	(20.0%)	(8.0%)	(0.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Croissant, pastries, bolycao, donuts and homemade cakes (one or a slice)	**10**	5	2	4	1	2	0	0	0	0	1	0	0	0	0	0	0	0
	(40.0%)	(20.0%)	(8.0%)	(16.0%)	(4.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Chocolate in tablet or powder (three squares or one tablespoon)	**13**	3	2	2	1	1	1	0	0	0	1	1	0	0	0	0	0	0
	(52.0%)	(12.0%)	(8.0%)	(8.0%)	(4.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Chocolate snacks such as Mars, Twix, Kit Kat, etc. (one)	**15**	3	4	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	(60.0%)	(12.0%)	(16.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Marmalade, jam, jelly, honey (one tablespoon)	**16**	3	1	3	0	0	0	0	0	1	1	0	0	0	0	0	0	0
	(64.0%)	(12.0%)	(4.0%)	(12.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Sugar (one dessert spoon or a package)	**12**	1	5	2	0	4	0	0	0	0	1	0	0	0	0	0	0	0
	(48.0%)	(4.0%)	(20.0%)	(8.0%)	(0.0%)	(16.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

Table S11. Usual nutritional intake: distribution of frequency, annual and seasonal of sweets and pastry.

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	1 - 3 / month	1 / week	2 - 4 / week	5 - 6 / week	1 / day	2 - 3 / day	4 - 5 / day	≥ 6 / day	Never / less than once a month	1 - 3 / month	1 / week	2 - 4 / week	5 - 6 / week	1 / day	2 - 3 / day	4 - 5 / day	≥ 6 / day
White cabbage and savoy cabbage (half cup)	**14**	2	3	4	0	0	2	0	0	0	0	0	0	0	0	0	0	0
	(56.0%)	(8.0%)	(12.0%)	(16.0%)	(0.0%)	(0.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Cabbage bunch and "tronchuda" (half cup)	**17**	2	3	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	(68.0%)	(8.0%)	(12.0%)	(8.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Kale (half cup)	**15**	5	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	(60.0%)	(20.0%)	(16.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Broccoli (half cup)	**12**	5	5	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	(48.0%)	(20.0%)	(20.0%)	(8.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Cauliflower and Brussels sprout (half cup)	**15**	3	3	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0
	(60.0%)	(12.0%)	(12.0%)	(4.0%)	(4.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Greens, turnip greens and spinach (half cup)	**18**	1	1	3	0	0	1	0	0	0	0	1	0	0	0	0	0	0
	(72.0%)	(4.0%)	(4.0%)	(12.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Green beans (half cup)	**13**	3	3	4	0	0	1	0	0	0	0	1	0	0	0	0	0	0
	(52.0%)	(12.0%)	(12.0%)	(16.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Lettuce and watercress (half cup)	**15**	1	1	4	0	2	1	0	0	0	0	1	0	0	0	0	0	0
	(60.0%)	(4.0%)	(4.0%)	(16.0%)	(0.0%)	(8.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Onion (half, an average)	**13**	2	2	4	1	1	1	0	0	0	0	0	0	1	0	0	0	0
	(52.0%)	(8.0%)	(8.0%)	(16.0%)	(4.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

Table S12. Usual nutritional intake: distribution of frequency, annual and seasonal of vegetables.

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**	**Never / less than once a month**	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**
Carrot (average)	**7**	3	4	**7**	2	1	1	0	0	0	0	0	0	0	0	0	0	0
	(28.0%)	(12.0%)	(16.0%)	**(28.0%)**	(8.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Turnip (an average)	**15**	2	2	4	1	0	0	0	0	0	0	1	0	0	0	0	0	0
	(60.0%)	(8.0%)	(8.0%)	(16.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Fresh tomatoes (three slices)	**14**	3	0	4	0	1	0	1	0	0	0	0	2	0	0	0	0	0
	(56.0%)	(12.0%)	(0.0%)	(16.0%)	(0.0%)	(4.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Pepper (six slices)	**23**	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	(92.0%)	(0.0%)	(0.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Cucumber (1/4 medium)	**18**	1	0	1	0	0	0	0	0	1	0	0	4	0	0	0	0	0
	(72.0%)	(4.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(16.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Beans and chickpeas (a cup or half plate)	**11**	3	8	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(44.0%)	(12.0%)	(32.0%)	(12.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Peas and fava beans (half cup or 1/4 dish)	**11**	5	5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(44.0%)	(20.0%)	(20.0%)	(16.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

Table S13. Usual nutritional intake: distribution of frequency, annual and seasonal of vegetables (cont.).

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**	**Never / less than once a month**	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**
Apple and pear (an average)	**7**	3	4	4	1	3	1	0	0	0	0	1	0	1	0	0	0	0
	(28.0%)	(12.0%)	(16.0%)	(16.0%)	(4.0%)	(12.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Orange and tangerines (an average, two medium)	**15**	3	2	2	0	2	0	0	0	0	0	1	0	0	0	0	0	0
	(60.0%)	(12.0%)	(8.0%)	(8.0%)	(0.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Banana (average)	**12**	2	5	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	(48.0%)	(8.0%)	(20.0%)	(20.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Kiwi (average)	**15**	2	4	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0
	(60.0%)	(8.0%)	(16.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Strawberries (one cup)	**12**	0	2	2	0	0	0	0	0	1	0	4	2	1	0	0	0	0
	(50.0%)	(0.0%)	(8.3%)	(8.3%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.2%)	(0.0%)	(16.7%)	(8.3%)	(4.2%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Cherries (a cup)	**15**	0	1	0	0	0	0	0	0	1	0	3	3	1	0	0	0	0
	(62.5%)	(0.0%)	(4.2%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.2%)	(0.0%)	(12.5%)	(12.5%)	(4.2%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Plum (three average)	**11**	1	1	2	0	0	0	0	0	2	0	4	1	1	0	0	0	0
	(47.8%)	(4.3%)	(4.3%)	(8.7%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(8.7%)	(0.0%)	(17.4%)	(4.3%)	(4.3%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Melon and watermelon (an average slice)	**12**	0	1	3	0	0	0	0	0	1	0	5	1	0	1	0	0	0
	(50.0%)	(0.0%)	(4.2%)	(12.5%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.2%)	(0.0%)	(20.8%)	(4.2%)	(0.0%)	(4.2%)	(0.0%)	(0.0%)	(0.0%)

Table S14. Usual nutritional intake: distribution of frequency, annual and seasonal of fruits.

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	1 - 3 / month	1 / week	2 - 4 / week	5 - 6 / week	1 / day	2 - 3 / day	4 - 5 / day	≥ 6 / day	Never / less than once a month	1 - 3 / month	1 / week	2 - 4 / week	5 - 6 / week	1 / day	2 - 3 / day	4 - 5 / day	≥ 6 / day
Persimmon (average)	**16**	1	1	0	0	0	0	0	0	2	1	3	0	0	0	0	0	0
	(66.7%)	(4.2%)	(4.2%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(8.3%)	(4.2%)	(12.5%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Fresh figs, loquats and apricots (average three)	**19**	1	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
	(79.2%)	(4.2%)	(4.2%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.2%)	(4.2%)	(4.2%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Fresh grapes (an average bunch)	**13**	4	3	1	0	0	0	0	0	1	0	2	1	0	0	0	0	0
	(52.0%)	(16.0%)	(12.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(8.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Canned fruits such as peach and pineapple (two halves or slices)	**18**	2	3	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	(72.0%)	(8.0%)	(12.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Almonds, hazelnuts, walnuts, peanuts, pistachio, etc. (half cup peeled)	**20**	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(80.0%)	(16.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Olives (six units)	**19**	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(76.0%)	(20.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

Table S15. Usual nutritional intake: distribution of frequency, annual and seasonal of fruits (cont.)

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**	**Never / less than once a month**	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**
Wine (a glass of 250ml)	**25**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(100.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Beer (a bottle or a can)	**25**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(100.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
White spirits such as whiskey, rum, brandy, etc. (one 40 ml cup)	**25**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(100.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Coke, pepsi or other (a bottle or a can)	**19**	1	0	3	0	1	1	0	0	0	0	0	0	0	0	0	0	0
	(76.0%)	(4.0%)	(0.0%)	(12.0%)	(0.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Ice tea (a bottle or a can)	**9**	3	3	6	1	0	3	0	0	0	0	0	0	0	0	0	0	0
	(36.0%)	(12.0%)	(12.0%)	(24.0%)	(4.0%)	(0.0%)	(12.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Other soft drinks, fruit juices or nectars packed (a bottle or a cup)	6	**7**	3	1	1	5	1	1	0	0	0	0	0	0	0	0	0	0
	(24.0%)	**(28.0%)**	(12.0%)	(4.0%)	(4.0%)	(20.0%)	(4.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Coffee, including added to other beverages (coffee cup)	**20**	2	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	(80.0%)	(8.0%)	(4.0%)	(4.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Black and green tea (one cup)	**23**	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	(92.0%)	(4.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

Table S16. Usual nutritional intake: distribution of frequency, annual and seasonal of drinks.

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**	**Never / less than once a month**	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**
Croquettes, patties, codfish balls, etc. (three units)	**12**	6	4	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	(48.0%)	(24.0%)	(16.0%)	(8.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Mayonnaise (one tablespoon)	**19**	2	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(76.0%)	(8.0%)	(12.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Tomato sauce, ketchup (one tablespoon)	**19**	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(76.0%)	(12.0%)	(8.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Pizza (half average pizza)	**13**	10	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(52.0%)	(40.0%)	(8.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Hamburger (average)	10	**11**	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	(40.0%)	**(44.0%)**	(12.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Vegetable soup (a dish)	2	2	0	**6**	4	5	5	0	1	0	0	0	0	0	0	0	0	0
	(8.0%)	(8.0%)	(0.0%)	**(24.0%)**	(16.0%)	(20.0%)	(20.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Soup (a dish)	**17**	5	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(68.0%)	(20.0%)	(8.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

Table S17. Usual nutritional intake: distribution of frequency, annual and seasonal of other foods.

Frequency of ingestion	Annual									Seasonal								
	Never / less than once a month	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**	**Never / less than once a month**	**1 - 3 / month**	**1 / week**	**2 - 4 / week**	**5 - 6 / week**	**1 / day**	**2 - 3 / day**	**4 - 5 / day**	**≥ 6 / day**
Cerelac baby food (a dish)	**24**	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	(96.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Chia seeds (a dessert spoon)	**24**	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	(96.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(4.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
"Alheira" (one medium)	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(0.0%)	**(100.0%)**	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Candies (one)	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(0.0%)	(0.0%)	**(100.0%)**	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Lupins (a small dish)	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(0.0%)	(0.0%)	(0.0%)	**(100.0%)**	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Sweet potatoes (an average)	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(0.0%)	(0.0%)	(0.0%)	**(100.0%)**	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Soymilk (a cup of 200 ml)	0	0	**1**	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
	(0.0%)	(0.0%)	**(50.0%)**	(0.0%)	**(50.0%)**	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Soy dessert (a unit of 120g)	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
	(0.0%)	(0.0%)	(0.0%)	(0.0%)	**(100.0%)**	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
Soybean cakes and biscuits (biscuits 3 to 4)	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
	(0.0%)	(0.0%)	(0.0%)	(0.0%)	**(100.0%)**	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

Table S18. Usual nutritional intake: distribution of frequency, annual and seasonal of other foods mentioned by individuals.

Nutrient	Mean	SD	Median	Minimum	Maximum
Energy (Kcal)	1974.6	742.2	1889.6	395.2	3910.1
Energy (Kcal/Kg/day)	*77.5*	*38.7*	*72.6*	*13.4*	*179.1*
Protein (g)	97.8	31.8	96.9	23.5	160.6
Protein (% / energy)	*20.5*	*3.7*	*20.2*	*14.2*	*28.7*
Fat (g)	69.9	24.5	74.4	14.1	120.4
Fat (% / energy)	32.4	5.4	32.1	21.9	46.7
Saturated fat (g)	22.7	9.1	21.0	3.3	36.5
Monounsaturated fat (g)	29.0	10.9	28.8	6.8	57.2
Polyunsaturated fat (g)	12.2	4.2	12.0	2.5	20.2
Omega 3 fatty acid (g)	1.5	0.6	1.3	0.4	3.4
Omega 6 fatty acid (g)	8.8	3.6	8.3	1.8	17.6
Cholesterol (g)	316.6	144.3	301.1	60.1	728.6
Carbohydrate (g)	245.4	124.2	226.6	44.6	626.6
Carbohydrate (% / energy)	*48.3*	*6.9*	*46.7*	*38.9*	*64.1*
Sugars (g)	109.9	75.5	100.3	11.3	364.4
Dietary fiber (g)	20.6	15.8	16.1	5.1	76.2

Table S19. Usual nutritional intake in 25 MPS patients: energy and macronutrients.

N=25	Low		Adequate		High			
	n	%	n	%	n	%	Mean	SD
Energy	14	56.0%	4	16.0%	7	28.0%	102.5	59.3

Table S20. *Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher energy than recommendations. Mean (and SD) are presented in % of EAR.*

		0-4 y		5-11 y		12-18 y		>18 y	
		n	%	n	%	n	%	n	%
Energy	Low	0	0.0%	4	44.4%	6	66.7%	4	80.0%
(% of EAR)	Adequate	1	50.0%	1	11.1%	2	22.2%	0	0.0%
	High	1	50.0%	4	44.4%	1	11.1%	1	20.0%

Table S21. *Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher energy than recommendations, according to age group.*

	0-4 y		5-11 y		12-18 y		>18 y	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy (% of EAR)	118.4	37.1	115.4	55.7	84.9	39.5	104.5	101.0

Table S22. Usual nutritional intake: percentage adequacy of energy intake, according to age group.

		MPS I		MPS II		MPS III		MPS IV		MPS VI		MPS VII	
		n	%	n	%	n	%	n	%	n	%	n	%
Energy	Low	1	20.0%	1	25.0%	2	50.0%	2	100%	7	77.8%	1	100%
(% of EAR)	Adequate	2	40.0%	1	25.0%	0	0.0%	0	0.0%	1	11.1%	0	0.0%
	High	2	40.0%	2	50.0%	2	50.0%	0	0.0%	1	11.1%	0	0.0%

Table S23. Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher energy than recommendations, according to MPS type.

	MPS I		MPS II		MPS III		MPS IV		MPS VI		MPS VII	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD [a]
Energy (% of EAR)	135.9	85.8	124.1	45.8	118.7	84.8	70.5	20.2	76.4	34.4	83.2	.

Table S24. Usual nutritional intake: percentage adequacy of energy intake, according to MPS type ([a] n=1).

Nutrient	Low		Adequate		High		Mean	SD
	n	%	n	%	n	%		
Protein	8	32.0%	11	44.0%	6	24.0%	102.6	27.3
Carbohydrate	16	64.0%	7	28.0%	2	8.0%	87.9	12.6
Fiber	19	76.0%	3	12.0%	3	12.0%	74.7	61.6
Fat	3	12.0%	13	52.0%	9	36.0%	110.6	20.0
Omega 3 fatty acid	18	72.0%	6	24.0%	1	4.0%	77.7	25.4
Omega 6 fatty acid	23	92.0%	2	8.0%	0	0.0%	55.4	17.4

Table S25. Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher amounts of macronutrients than recommendations. Mean (and SD) are presented in % of EAR.

Nutrient		0-4 y		5-11 y		12-18 y		>18 y	
		n	%	n	%	n	%	n	%
Protein	Low	0	0.0%	5	55.6%	1	11.1%	2	40.0%
	Adequate	1	50.0%	4	44.4%	5	55.6%	1	20.0%
	High	1	50.0%	0	0.0%	3	33.3%	2	40.0%
Carbohydrate	Low	2	100.0%	2	22.2%	7	77.8%	5	100.0%
	Adequate	0	0.0%	5	55.6%	2	22.2%	0	0.0%
	High	0	0.0%	2	22.2%	0	0.0%	0	0.0%
Fat	Low	0	0.0%	2	22.2%	0	0.0%	1	20.0%
	Adequate	2	100.0%	5	55.6%	5	55.6%	1	20.0%
	High	0	0.0%	2	22.2%	4	44.4%	3	60.0%
Omega 3 fatty acid	Low	2	100.0%	7	77.8%	6	66.7%	3	60.0%
	Adequate	0	0.0%	2	22.2%	3	33.3%	1	20.0%
	High	0	0.0%	0	0.0%	0	0.0%	1	20.0%
Omega 6 fatty acid	Low	2	100.0%	9	100.0%	8	88.9%	4	80.0%
	Adequate	0	0.0%	0	0.0%	1	11.1%	1	20.0%
	High	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Table S26. Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher amounts of macronutrients than recommendations, according to age group.

Nutrient (% of EAR)	0-4 y		5-11 y		12-18 y		>18 y	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Protein	127.5	24.7	89.1	13.6	102.8	12.0	116.7	51.8
Carbohydrate	78.2	10.3	97.2	13.2	85.5	10.1	79.6	5.4
Fat	106.7	0.0	100.7	16.7	113.4	12.8	125.0	32.0
Omega 3 fatty acid	70.5	11.1	68.4	21.3	80.1	18.4	93.0	41.8
Omega 6 fatty acid	39.5	5.8	50.2	15.8	60.0	18.8	62.6	17.1

Table S27. Usual nutritional intake: percentage adequacy of macronutrients intake, according to age group.

Nutrient		MPS I		MPS II		MPS III		MPS IV		MPS VI		MPS VII	
		n	%	n	%	n	%	n	%	n	%	n	%
Protein	Low	0	0.0%	1	25.0%	1	25.0%	1	50.0%	5	55.6%	0	0.0%
	Adequate	3	60.0%	2	50.0%	1	25.0%	1	50.0%	3	33.3%	1	100%
	High	2	40.0%	1	25.0%	2	50.0%	0	0.0%	1	11.1%	0	0.0%
Carbohydrate	Low	5	100%	2	50.0%	2	50.0%	1	50.0%	6	66.7%	0	0.0%
	Adequate	0	0.0%	1	25.0%	1	25.0%	1	50.0%	3	33.3%	1	100%
	High	0	0.0%	1	25.0%	1	25.0%	0	0.0%	0	0.0%	0	0.0%
Fat	Low	1	20.0%	1	25.0%	1	25.0%	0	0.0%	0	0.0%	0	0.0%
	Adequate	2	40.0%	3	75.0%	2	50.0%	1	50.0%	4	44.4%	1	100%
	High	2	40.0%	0	0.0%	1	25.0%	1	50.0%	5	55.6%	0	0.0%
Omega 3 fatty acid	Low	3	60.0%	3	75.0%	3	75.0%	1	50.0%	7	77.8%	1	100%
	Adequate	1	20.0%	1	25.0%	1	25.0%	1	50.0%	2	22.2%	0	0.0%
	High	1	20.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Omega 6 fatty acid	Low	5	100%	4	100%	4	100%	2	100%	7	77.8%	1	100%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	22.2%	0	0.0%
	High	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Table S28. Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher amounts of macronutrients than recommendations, according to MPS type.

Nutrient (% of EAR)	MPS I		MPS II		MPS III		MPS IV		MPS VI		MPS VII	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD[a]
Protein	130.4	43.4	100.3	14.4	100.0	22.7	92.8	24.4	91.9	17.3	100.0	.
Carbohydrate	81.8	7.3	96.8	16.1	90.5	17.4	92.7	20.6	84.2	9.4	96.4	.
Fat	108.5	14.9	97.2	16.4	105.8	16.6	108.9	20.4	122.2	23.3	93.3	.
Omega 3 fatty acid	94.9	40.4	80.3	20.3	69.4	22.8	78.3	28.0	72.1	19.9	63.8	.
Omega 6 fatty acid	48.1	9.6	48.6	10.7	47.44	9.4	52.6	14.7	63.2	21.9	85.7	

Table S29. Usual nutritional intake: percentage adequacy of macronutrients intake, according to MPS type ([a] n=1).

Nutrient	Mean	SD	Median	Minimum	Maximum
Vitamin A (total. RE)	2571.8	2564.0	1670.5	122.6	11378.3
Vitamin B_1 (mg)	1.6	0.7	1.5	0.3	3.9
Vitamin B_2 (mg)	2.2	1.2	2.2	0.3	5.7
Vitamin B_3 (mg)	23.3	8.3	22.9	6.4	50.7
Vitamin B_6 (mg)	2.3	1.1	2.1	0.7	5.8
Biotin (µg)	10.8	9.6	8.0	0.0	45.8
Vitamin B_{12} (µg)	12.2	8.8	9.9	2.8	41.1
Folate (µg)	314.1	201.7	296.3	47.9	908.4
Pantothenic acid (mg)	4.8	2.3	4.7	1.0	10.5
Vitamin C (mg)	124.9	125.2	86.5	22.0	605.3
Vitamin D (µg)	4.7	2.3	4.8	1.4	10.1
Vitamin E (mg)	8.1	4.0	6.9	2.1	17.6
Vitamin K (µg)	16.7	17.1	11.5	0.0	79.3

Table S30. Usual nutritional intake in 25 MPS patients: vitamins (RE=retinol equivalents).

Nutrient	Low		Adequate		High		Mean	SD
	n	%	n	%	n	%		
Vitamin A (total)	2	8.0%	0	0.0%	23	92.0%	646.2	833.3
Vitamin B_1	2	8.0%	1	4.0%	22	88.0%	227.1	123.4
Vitamin B_2	2	8.0%	0	0.0%	23	92.0%	305.5	201.7
Vitamin B_3	1	4.0%	1	4.0%	23	92.0%	264.1	123.0
Vitamin B_6	2	8.0%	0	0.0%	23	92.0%	316.2	242.8
Biotin	20	80.0%	2	8.0%	3	12.0%	60.6	59.2
Vitamin B_{12}	0	0.0%	0	0.0%	25	100.0%	825.2	624.0
Folate	9	36.0%	4	16.0%	12	48.0%	139.2	142.9
Pantothenic acid	9	36.0%	4	16.0%	12	48.0%	127.9	86.9
Vitamin C	4	16.0%	5	20.0%	16	64.0%	441.7	914.9
Vitamin D	24	96.0%	1	4.0%	0	0.0%	47.1	22.6
Vitamin E	13	52.0%	3	12.0%	9	36.0%	81.9	45.0
Vitamin K	24	96.0%	1	4.0%	0	0.0%	26.3	28.2

Table S31. Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher amounts of vitamins than recommendations. Mean (and SD) are presented in % of EAR.

Nutrient		0-4 y		5-11 y		12-18 y		>18 y	
		n	%	n	%	n	%	n	%
Vitamin A (total)	Low	0	0.0%	0	0.0%	1	11.1%	1	20.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	2	100.0%	9	100.0%	8	88.9%	4	80.0%
Vitamin B_1	Low	0	0.0%	0	0.0%	1	11.1%	1	20.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	1	20.0%
	High	2	100.0%	9	100.0%	8	88.9%	3	60.0%
Vitamin B_2	Low	0	0.0%	0	0.0%	1	11.1%	1	20.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	2	100.0%	9	100.0%	8	88.9%	4	80.0%
Vitamin B_3	Low	0	0.0%	0	0.0%	1	11.1%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	1	20.0%
	High	2	100.0%	9	100.0%	8	88.9%	4	80.0%
Vitamin B_6	Low	0	0.0%	0	0.0%	1	11.1%	1	20.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	2	100.0%	9	100.0%	8	88.9%	4	80.0%
Biotin	Low	0	0.0%	8	88.9%	8	88.9%	4	80.0%
	Adequate	0	0.0%	1	11.1%	1	11.1%	0	0.0%
	High	2	100.0%	0	0.0%	0	0.0%	1	20.0%
Vitamin B_{12}	Low	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	2	100.0%	9	100.0%	9	100.0%	5	100.0%
Folate	Low	0	0.0%	2	22.2%	5	55.6%	2	40.0%
	Adequate	1	50.0%	1	11.1%	1	11.1%	1	20.0%
	High	1	50.0%	6	66.7%	3	33.3%	2	40.0%
Pantothenic acid	Low	0	0.0%	3	33.3%	4	44.4%	2	40.0%
	Adequate	0	0.0%	0	0.0%	3	33.3%	1	20.0%
	High	2	100.0%	6	66.7%	2	22.2%	2	40.0%
Vitamin C	Low	0	0.0%	0	0.0%	2	22.2%	2	40.0%
	Adequate	0	0.0%	1	11.1%	3	33.3%	1	20.0%
	High	2	100.0%	8	88.9%	4	44.4%	2	40.0%
Vitamin D	Low	2	100.0%	9	100.0%	8	88.9%	5	100.0%
	Adequate	0	0.0%	0	0.0%	1	11.1%	0	0.0%
	High	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Vitamin E	Low	1	50.0%	3	33.3%	5	55.6%	4	80.0%
	Adequate	1	50.0%	1	11.1%	1	11.1%	0	0.0%
	High	0	0.0%	5	55.6%	3	33.3%	1	20.0%
Vitamin K	Low	1	50.0%	9	100.0%	9	100.0%	5	100.0%
	Adequate	1	50.0%	0	0.0%	0	0.0%	0	0.0%
	High	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Table S32. Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher amounts of vitamins than recommendations, according to age group.

Nutrient (% of EAR)	0-4 y		5-11 y		12-18 y		>18 y	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Vitamin A (total)	647.0	229.6	641.6	525.1	363.0	254.3	1164.0	1719.5
Vitamin B_1	259.4	102.7	268.9	119.7	198.6	100.2	190.3	179.2
Vitamin B_2	629.4	10.4	315.4	130.7	233.9	146.1	287.2	320.4
Vitamin B_3	280.0	98.0	282.0	108.7	256.2	116.5	240.0	189.4
Vitamin B_6	345.0	103.2	339.8	165.3	262.9	169.3	358.4	478.4
Biotin	203.8	29.3	58.6	27.1	37.4	31.0	48.5	76.0
Vitamin B_{12}	1476.8	815.6	728.3	391.3	661.5	479.1	1033.9	1027.8
Folate	106.2	15.4	146.0	67.4	99.3	64.3	212.1	306.9
Pantothenic acid	219.1	12.6	132.2	66.8	92.8	42.1	146.8	159.2
Vitamin C	213.9	129.4	346.8	222.5	272.7	349.2	1007.9	2040.6
Vitamin D	44.1	26.3	41.4	17.2	53.2	27.2	47.6	25.9
Vitamin E	67.1	37.6	101.1	40.8	82.3	50.9	52.7	35.9
Vitamin K	97.3	12.1	22.5	12.2	17.4	15.8	20.6	34.2

Table S33. Usual nutritional intake: percentage adequacy of vitamins intake, according to age group.

Nutrient		MPS I		MPS II		MPS III		MPS IV		MPS VI		MPS VII	
		n	%	n	%	n	%	n	%	n	%	n	%
Vitamin A (total)	Low	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	22.2%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	5	100%	4	100%	4	100%	2	100%	7	77.8%	1	100%
Vitamin B_1	Low	0	0.0%	0	0.0%	1	25.0%	0	0.0%	1	11.1%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	11.1%	0	0.0%
	High	5	100%	4	100%	3	75.0%	2	100%	7	77.8%	1	100%
Vitamin B_2	Low	0	0.0%	0	0.0%	1	25.0%	0	0.0%	1	11.1%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	5	100%	4	100%	3	75.0%	2	100%	8	88.9%	1	100%
Vitamin B_3	Low	0	0.0%	0	0.0%	1	25.0%	0	0.0%	0	0.0%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	11.1%	0	0.0%
	High	5	100%	4	100%	3	75.0%	2	100%	8	88.9%	1	100%
Vitamin B_6	Low	0	0.0%	0	0.0%	1	25.0%	0	0.0%	1	11.1%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	5	100%	4	100%	3	75.0%	2	100%	8	88.9%	1	100%
Biotin	Low	2	40.0%	3	75.0%	3	75.0%	2	100%	9	100%	1	100%
	Adequate	0	0.0%	1	25.0%	1	25.0%	0	0.0%	0	0.0%	0	0.0%
	High	3	60.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Vitamin B_{12}	Low	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	5	100%	4	100%	4	100%	2	100%	9	100%	1	100%
Folate	Low	1	20.0%	1	25.0%	1	25.0%	0	0.0%	6	66.7%	0	0.0%
	Adequate	1	20.0%	0	0.0%	1	25.0%	1	50.0%	1	11.1%	0	0.0%
	High	3	60.0%	3	75.0%	2	50.0%	1	50.0%	2	22.2%	1	100%
Pantothenic acid	Low	1	20.0%	1	25.0%	1	25.0%	1	50.0%	5	55.6%	0	0.0%
	Adequate	1	20.0%	0	0.0%	0	0.0%	1	50.0%	2	22.2%	0	0.0%
	High	3	60.0%	3	75.0%	3	75.0%	0	0.0%	2	22.2%	1	100%
Vitamin C	Low	0	0.0%	1	25.0%	1	25.0%	0	0.0%	2	22.2%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	5	55.6%	0	0.0%
	High	5	100%	3	75.0%	3	75.0%	2	100%	2	22.2%	1	100%
Vitamin D	Low	5	100%	3	75.0%	4	100%	2	100%	9	100%	1	100%
	Adequate	0	0.0%	1	25.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Vitamin E	Low	3	60.0%	1	25.0%	2	50.0%	1	50.0%	6	66.7%	0	0.0%
	Adequate	2	40.0%	0	0.0%	0	0.0%	0	0.0%	1	11.1%	0	0.0%
	High	0	0.0%	3	75.0%	2	50.0%	1	50.0%	2	22.2%	1	100%
Vitamin K	Low	4	80.0%	4	100%	4	100%	2	100%	9	100%	1	100%
	Adequate	1	20.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Table S34. Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher amounts of vitamins than recommendations, according to MPS type.

Nutrient	MPS I		MPS II		MPS III		MPS IV		MPS VI		MPS VII	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD[a]
Vitamin A (total)	1266.2	1655.8	993.7	660.6	407.8	266.7	383.5	139.1	315.9	224.5	608.1	.
Vitamin B_1	284.9	143.8	299.0	124.1	251.3	189.3	185.7	36.3	155.6	67.2	280.0	.
Vitamin B_2	511.9	279.2	347.8	177.5	305.3	223.0	196.7	5.9	193.0	88.5	336.3	.
Vitamin B_3	327.2	151.0	377.2	115.1	262.7	154.6	235.8	63.9	187.6	62.0	247.6	.
Vitamin B_6	478.3	419.3	466.0	207.3	327.6	210.7	260.3	49.1	170.3	58.3	287.5	.
Biotin	130.7	93.0	56.6	31.1	63.1	43.4	21.4	15.1	28.8	21.2	79.6	.
Vitamin B_{12}	1297.8	961.0	1026.1	573.4	827.7	582.6	683.4	185.8	560.5	418.8	316.0	.
Folate	238.7	291.8	190.5	77.3	110.0	70.0	126.4	26.0	71.8	33.8	187.0	.
Pantothenic acid	204.6	142.4	147.9	56.2	132.6	100.5	89.9	15.4	83.8	32.2	118.3	.
Vitamin C	1104.0	1987.3	579.7	435.3	330.3	303.0	326.6	188.1	114.2	73.5	202.6	.
Vitamin D	52.4	25.4	67.5	33.1	40.4	24.1	47.2	17.0	40.2	14.3	28.5	.
Vitamin E	66.7	32.5	126.5	57.1	79.5	47.8	92.8	40.0	65.3	40.3	117.1	.
Vitamin K	60.3	44.3	20.5	13.8	22.6	19.5	8.6	3.9	13.6	12.2	43.5	.

Table S35. Usual nutritional intake: percentage adequacy of vitamins intake, according to MPS type ([a] n=1).

Nutrient	Mean	SD	Median	Minimum	Maximum
Calcium (mg)	991.7	605.4	767.3	86.5	2622.7
Copper (mg)	1.7	0.9	1.5	0.4	4.9
Iron (mg)	14.8	6.5	14.8	3.0	38.1
Magnesium (mg)	313.2	146.2	299.4	67.1	770.9
Manganesium (µg)	3.2	2.0	2.9	0.6	9.8
Phosphorus (mg)	1430.4	577.0	1436.4	245.4	2725.6
Potassium (mg)	3556.2	1803.1	3304.9	847.3	9007.5
Selenium (µg)	96.8	39.3	107.6	21.2	198.7
Sodium (from food + added. mg)	3521.0	1358.4	3469.0	1126.6	7650.0
Zinc (mg)	12.1	4.8	12.1	2.3	24.9

Table S36. Usual nutritional intake in 25 MPS patients: minerals and oligoelements.

Nutrient	Low		Adequate		High		Mean	SD
	n	%	n	%	n	%		
Calcium	11	44.0%	4	16.0%	10	40.0%	114.6	88.6
Copper	2	8.0%	0	0.0%	23	92.0%	335.4	225.6
Iron	2	8.0%	1	4.0%	22	88.0%	266.3	146.4
Magnesium	7	28.0%	5	20.0%	13	52.0%	179.9	180.4
Manganesium	25	100.0%	0	0.0%	0	0.0%	0.2	0.1
Phosphorus	3	12.0%	0	0.0%	22	88.0%	220.2	126.0
Potassium	17	68.0%	4	16.0%	4	16.0%	83.6	49.8
Selenium	1	4.0%	1	4.0%	23	92.0%	288.6	154.1
Sodium (from food plus added)	2	8.0%	0	0.0%	23	92.0%	253.5	98.4
Zinc	2	8.0%	2	8.0%	21	84.0%	212.0	140.6

Table S37. Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher amounts of minerals and oligoelements than recommendations. Mean (and SD) are presented in % of EAR.

Nutrient		0-4 y		5-11 y		12-18 y		>18 y	
		n	%	n	%	n	%	n	%
Calcium	Low	0	0.0%	2	22.2%	6	66.7%	3	60.0%
	Adequate	0	0.0%	3	33.3%	1	11.1%	0	0.0%
	High	2	100.0%	4	44.4%	2	22.2%	2	40.0%
Copper	Low	0	0.0%	0	0.0%	1	11.1%	1	20.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	2	100.0%	9	100.0%	8	88.9%	4	80.0%
Iron	Low	0	0.0%	0	0.0%	1	11.1%	1	20.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	1	20.0%
	High	2	100.0%	9	100.0%	8	88.9%	3	60.0%
Magnesium	Low	0	0.0%	2	22.2%	2	22.2%	3	60.0%
	Adequate	0	0.0%	1	11.1%	4	44.4%	0	0.0%
	High	2	100.0%	6	66.7%	3	33.3%	2	40.0%
Manganesium	Low	2	100.0%	9	100.0%	9	100.0%	5	100.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Phosphorus	Low	0	0.0%	0	0.0%	2	22.2%	1	20.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	2	100.0%	9	100.0%	7	77.8%	4	80.0%
Potassium	Low	0	0.0%	6	66.7%	8	88.9%	3	60.0%
	Adequate	1	50.0%	2	22.2%	0	0.0%	1	20.0%
	High	1	50.0%	1	11.1%	1	11.1%	1	20.0%
Selenium	Low	0	0.0%	0	0.0%	1	11.1%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	1	20.0%
	High	2	100.0%	9	100.0%	8	88.9%	4	80.0%
Sodium (from food plus added)	Low	0	0.0%	0	0.0%	1	11.1%	1	20.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	2	100.0%	9	100.0%	8	88.9%	4	80.0%
Zinc	Low	0	0.0%	0	0.0%	1	11.1%	1	20.0%
	Adequate	0	0.0%	1	11.1%	0	0.0%	1	20.0%
	High	2	100.0%	8	88.9%	8	88.9%	3	60.0%

Table S38. Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher amounts of minerals and oligoelements than recommendations, according to age group.

Nutrient	0-4 y		5-11 y		12-18 y		>18 y	
(% of EAR)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Calcium	233.1	7.6	105.5	26.8	75.1	37.4	154.6	173.6
Copper	392.6	22.8	374.8	199.9	245.3	136.3	403.8	398.6
Iron	239.0	73.2	301.4	109.3	228.9	115.3	281.6	265.1
Magnesium	208.4	122.4	178.9	80.6	138.0	103.3	245.6	383.9
Manganesium	0.1	0.0	0.2	0.1	0.2	0.1	0.2	0.2
Phosphorus	360.8	148.7	209.9	83.9	184.0	119.4	247.8	182.2
Potassium	113.3	6.0	91.1	42.2	65.0	28.5	91.5	90.7
Selenium	324.0	4.7	350.9	167.7	275.2	159.3	186.7	113.2
Sodium (from food plus added)	257.3	59.6	282.7	108.3	222.6	66.1	255.0	144.7
Zinc	324.3	42.7	237.9	158.5	168.5	77.4	198.7	209.1

Table S39. Usual nutritional intake: percentage adequacy of minerals and oligoelements intake, according to age group.

Nutrient		MPS I		MPS II		MPS III		MPS IV		MPS VI		MPS VII	
		n	%	n	%	n	%	n	%	n	%	n	%
Calcium	Low	1	20.0%	2	50.0%	1	25.0%	1	50.0%	6	66.7%	0	0.0%
	Adequate	1	20.0%	0	0.0%	1	25.0%	1	50.0%	1	11.1%	0	0.0%
	High	3	60.0%	2	50.0%	2	50.0%	0	0.0%	2	22.2%	1	100%
Copper	Low	0	0.0%	0	0.0%	1	25.0%	0	0.0%	1	11.1%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	5	100%	4	100%	3	75.0%	2	100%	8	88.9%	1	100%
Iron	Low	0	0.0%	0	0.0%	1	25.0%	0	0.0%	1	11.1%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	11.1%	0	0.0%
	High	5	100%	4	100%	3	75.0%	2	100%	7	77.8%	1	100%
Magnesium	Low	0	0.0%	1	25.0%	1	25.0%	1	50.0%	4	44.4%	0	0.0%
	Adequate	1	20.0%	0	0.0%	1	25.0%	0	0.0%	3	33.3%	0	0.0%
	High	4	80.0%	3	75.0%	2	50.0%	1	50.0%	2	22.2%	1	100%
Manganesium	Low	5	100%	4	100%	4	100%	2	100%	9	100%	1	100%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Phosphorus	Low	0	0.0%	0	0.0%	1	25.0%	0	0.0%	2	22.2%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	5	100%	4	100%	3	75.0%	2	100%	7	77.8%	1	100%
Potassium	Low	2	40.0%	2	50.0%	2	50.0%	2	100%	8	88.9%	1	100%
	Adequate	1	20.0%	0	0.0%	2	50.0%	0	0.0%	1	11.1%	0	0.0%
	High	2	40.0%	2	50.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Selenium	Low	0	0.0%	0	0.0%	1	25.0%	0	0.0%	0	0.0%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	11.1%	0	0.0%
	High	5	100%	4	100%	3	75.0%	2	100%	8	88.9%	1	100%
Sodium (from food plus added)	Low	0	0.0%	0	0.0%	1	25.0%	0	0.0%	1	11.1%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	High	5	100%	4	100%	3	75.0%	2	100%	8	88.9%	1	100%
Zinc	Low	0	0.0%	0	0.0%	1	25.0%	0	0.0%	1	11.1%	0	0.0%
	Adequate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	22.2%	0	0.0%
	High	5	100%	4	100%	3	75.0%	2	100%	6	66.7%	1	100%

Table S40. Usual nutritional intake in 25 MPS patients: patients consuming lower, adequate and higher amounts of minerals and oligoelements than recommendations, according to MPS type.

Nutrient	MPS I		MPS II		MPS III		MPS IV		MPS VI		MPS VII	
(% of EAR)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD[a]
Calcium	215.2	157.5	110.0	42.6	87.2	53.2	72.9	28.8	82.1	31.4	114.5	.
Copper	465.1	359.8	470.5	219.3	331.4	242.8	283.0	68.6	226.7	112.1	246.3	.
Iron	329.7	236.2	379.8	118.7	240.1	143.8	262.0	4.3	187.8	82.7	316.5	.
Magnesium	324.9	346.7	240.8	126.2	158.2	117.7	119.8	50.4	95.8	43.7	174.9	.
Manganesium	0.2	0.2	0.3	0.2	0.2	0.1	0.1	0.0	0.1	0.1	0.3	.
Phosphorus	338.7	153.5	305.5	92.7	188.8	147.8	112.0	1.0	163.0	65.0	144.3	.
Potassium	116.5	79.0	116.6	56.0	77.2	40.3	70.2	5.5	57.4	21.2	74.5	.
Selenium	323.7	24.6	465.6	158.7	326.7	262.2	236.7	28.5	180.2	66.0	332.9	.
Sodium (from food plus added)	264.8	70.2	331.1	126.1	264.7	131.4	213.8	27.0	218.2	95.3	237.6	.
Zinc	311.7	167.3	248.5	83.9	266.0	252.6	132.2	19.3	138.4	50.1	173.1	.

*Table S41. Usual nutritional intake: percentage adequacy of minerals and oligoelements intake, according to MPS type (**a** n=1).*

Type of MPS	Mean	SD	Median	Minimum	Maximum
MPS I	17.3	5.4	14.9	12.3	26.1
	(-0.9)	*(1.8)*	*(-0.6)*	*(-2.8)*	*(0.7)*
MPS II	37.0	8.5	33.7	31.1	49.6
	(1.2)	*.*	*(1.2)*	*(1.2)*	*(1.2)*
MPS III	38.0	8.7	36.9	26.3	56.8
	(1.5)	*(0.4)*	*(1.5)*	*(1.2)*	*(1.8)*
MPS IV	27.0	10.0	22.5	20.0	38.5
MPS VI	27.8	6.9	29.0	16.8	41.5
	(-1.0)	*(1.8)*	*(-1.0)*	*(-2.3)*	*(0.3)*
MPS VII	29.5	.	29.5	29.5	29.5
	(-0.3)	*.*	*(-0.3)*	*(-0.3)*	*(-0.3)*
Total	30.2	10.3	29.5	12.3	56.8
	(-0.1)	*(1.6)*	*(0.3)*	*(-2.8)*	*(1.8)*

Table S42. Anthropometrics: weight, in Kg (z-score), according to MPS type.
Weight z-score was calculated for 9 patients (three MPS I; one MPS II; two MPS III; two MPS VI; one MPS VII).

Age group	Mean	SD	Median	Minimum	Maximum
0-4 years	13.6	1.8	13.6	12.3	14.9
	(0.0)	*(0.9)*	*(0.0)*	*(-0.6)*	*(0.7)*
5-11 years	28.5	8.3	29.5	14.8	41.5
	(-0.1)	*(1,8)*	*(0.3)*	*(-2.8)*	*(1.8)*
12-18 years	32.0	8.6	29.5	22.0	49.6
	(n.d.)	*(n.d.)*	*(n.d.)*	*(n.d.)*	*(n.d.)*
>18 years	34.2	12.0	32.0	18.3	56.8
	(n.d.)	*(n.d.)*	*(n.d.)*	*(n.d.)*	*(n.d.)*
Total	30.2	10.3	29.5	12.3	56.8
	(-0.1)	*(1.6)*	*(0.3)*	*(-2.8)*	*(1.8)*

Table S43. Anthropometrics: weight, in Kg (z-score) (n.d. – no determined).
Weight z-score was calculated for 9 patients (two 0-4 years; seven 5-11 years).

Type of MPS	Mean	SD	Median	Minimum	Maximum
MPS I	105.7	16.0	102.2	83.3	124.0
	(-3.3)	*(3.0)*	*(-3.3)*	*(-6.9)*	*(-0.3)*
MPS II	136.1	8.1	132.8	130.8	148.0
	(-2.8)	*(2.1)*	*(-2.9)*	*(-5.1)*	*(-0.2)*
MPS III	145.2	15.6	142.0	123.4	170.0
	(-0.7)	*(1.9)*	*(0.2)*	*(-3.8)*	*(1.5)*
MPS IV	110.1	15.2	106.5	97.0	126.7
	(-7.3)	*(2.5)*	*(-6.5)*	*(-10.1)*	*(-5.2)*
MPS VI	114.3	8.3	113.4	99.0	124.5
	(-6.2)	*(2.9)*	*(-7.3)*	*(-8.8)*	*(-0.1)*
MPS VII	103.0	.	103.0	103.0	103.0
	(-5.4)	*.*	*(-5.4)*	*(-5.4)*	*(-5.4)*
Total	124.2	20.5	123.7	83.3	170.0
	(-3.7)	*(3.3)*	*(-3.8)*	*(-10.1)*	*(1.5)*

Table S44. Anthropometrics: height, in cm (z-score), according to type of MPS.
Height z-score was calculated in 30 patients (five MPS I; four MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII).

Age group	Mean	SD	Median	Minimum	Maximum
0-4years	92.1	12.4	92.1	83.3	100.8
	(-0.3)	(0.0)	(-0.3)	(-0.4)	(-0.3)
5-11years	122.2	16.8	124.5	99.0	145.0
	(-1.7)	(2.7)	(-0.2)	(-6.5)	(1.5)
12-18years	127.7	12.8	126.7	109.0	148.0
	(-5.1)	(2.0)	(-5.2)	(-8.8)	(-2.0)
>18years	131.2	27.6	119.0	97.0	170.0
	(-5.6)	(3.9)	(-7.3)	(-10.1)	(0.9)
Total	124.2	20.5	123.7	83.3	170.0
	(-3.7)	(3.3)	(-3.8)	(-10.1)	(1.5)

Table S45. Anthropometrics: height, in cm (z-score).
Height z-score was calculated for 30 patients (two 0-4 years; eleven 5-11 years; nine 12-18 years; eight >18 years).

Type of MPS	Mean	SD	Median	Minimum	Maximum
MPS I	15.3	2.0	14.7	13.0	17.9
	(-1.1)	*(1.8)*	*(-0.8)*	*(-3.9)*	*(1.2)*
MPS II	19.7	2.0	19.1	18.1	22.6
	(0.4)	*(1.2)*	*(0.4)*	*(-1.1)*	*(1.8)*
MPS III	18.0	2.2	18.0	13.8	21.2
	(-0-3)	*(1.9)*	*(-0.3)*	*(-4.6)*	*(1.9)*
MPS IV	21.7	2.1	21.3	19.8	24.0
	(0.5)	*(0.5)*	*(0.6)*	*(-0.1)*	*(0.9)*
MPS VI	21.0	4.8	20.3	15.9	28.8
	(0.3)	*(1.2)*	*(0.6)*	*(-1.9)*	*(1.8)*
MPS VII	27.8	.	27.8	27.8	27.8
	(3.4)		*(3.4)*	*(3.4)*	*(3.4)*
Total	19.3	3.9	18.3	13.0	28.8
	(0.0)	*(1.6)*	*(0.4)*	*(-4.6)*	*(3.4)*

Table S46. Anthropometrics: BMI, in Kg/m² (z-score), according to MPS type.
BMI z-score was calculated in 30 patients (five MPS I; four MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII)

Age group	Mean	SD	Median	Minimum	Maximum
0-4 years	16.3	2.3	16.3	14.7	17.9
	(0.3)	(1.3)	(0.3)	(-0.6)	(1.2)
5-11 years	18.8	3.6	18.1	14.2	27.8
	(1.0)	(1.1)	(0.9)	(-0.8)	(3.4)
12-18 years	19.5	3.3	18.0	15.9	24.4
	(-0.4)	(1.0)	(-0.3)	(-1.9)	(1.0)
>18 years	20.5	5.3	20.7	13.0	28.8
	(-0.9)	(2.3)	(-0.3)	(-4.6)	(1.8)
Total	19.3	3.9	18.3	13.0	28.8
	(0.0)	(1.6)	(0.4)	(-4.6)	(3.4)

Table S47. Anthropometrics: BMI, in Kg/m² (z-score).
BMI z-score was calculated for 30 patients (two 0-4 years; eleven 5-11 years; nine 12-18 years; eight >18 years).

Group of disease	n	Body fat mass (%)					Body lean mass (%)					Total body water (%)				
		Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.
MPS I	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MPS II	4	21.5	16.0	20.7	3.9	40.7	16.2	10.4	18.0	2.9	25.7	62.4	11.3	66.4	46.3	70.4
MPS III	6	16.6	8.1	17.7	3.9	28.6	18.2	6.2	19.4	10.1	25.7	65.3	5.2	65.2	59.4	70.4
MPS IV	3	23.8	10.6	21.6	14.5	35.4	13.8	11.3	17.3	1.1	22.9	62.4	15.0	62.6	47.3	77.3
MPS VI	5	11.5	10.1	5.4	3.9	27.5	23.2	3.7	25.4	17.0	25.8	65.2	6.4	69.2	55.5	70.3
MPS VII	0	-	-	-	-		-		-	-	-	-		-	-	
Total	18	17.5	11.1	16.2	3.9	40.7	18.4	7.8	22.1	1.1	25.8	64.1	8.3	66.0	46.3	77.3

Table S48. Body composition, according to type of MPS (SD=standard deviation; Min=minimum; Max=maximum)

Group of disease	Phase angle (°)					
	n	Mean	SD	Median	Min.	Max.
MPS I	1	3.9	-	3.9	3.9	
MPS II	4	4.5	1.0	4.5	3.3	5.7
MPS III	7	4.5	1.0	4.7	3.0	5.5
MPS IV	3	4.4	0.5	4.2	4.0	4.9
MPS VI	6	5.0	0.7	4.8	4.3	6.2
MPS VII	0	-	-	-	-	-
Total	21	4.6	0.8	4.6	3.0	6.2

Table S49. Phase angle, according to type of MPS (SD=standard deviation; Min=minimum; Max=maximum)

		Walked distance (m)				
Type of MPS	**n**	**Mean**	**SD**	**Median**	**Minimum**	**Maximum**
MPS I	2	171.2	114.8	171.2	90.0	252.4
MPS II	2	554.5	149.2	554.5	449.0	660.0
MPS III	3	302.8	89.3	332.3	202.5	373.7
MPS IV	2	250.6	93.3	250.6	184.6	316.5
MPS VI	7	287.4	126.4	350.7	26.9	384.0
Total	16	304.6	147.1	324.4	26.9	660.0

Table S50. Six minutes walking test results.

Plasmatic proteins		n (%)	Mean	SD	Min	Max
Total protein (g/L)	Normal	25 (92.6%)	72.6	8.4	65.8	110.0
	Low	2 (7.4%)	64.0	0.0	64.0	64.0
	High	0 (0.0%)	.	.	.	.
	Total	27 (100.0%)	71.9	8.4	64.0	110.0
Albumin (g/L)	Normal	27 (87.1%)	42.9	2.9	36.5	48.8
	Low	2 (6.5%)	35. 5	1.6	34.3	36.6
	High	2 (6.5%)	51.5 7		51.0	52.0
	Total	31 (100.0%)	43.0	4.0	34.3	52.0
Ferritin (pmol/L)	Normal	30 (100.0%)	128.2	66.9	47.0	330.0
	Low	0 (0.0%)	.	.	.	.
	High	0 (0.0%)	.	.	.	.
	Total	30 (100.0%)	128.2	66.9	47.0	330.0
Pre-albumin (mg/L)	Normal	11 (40.7%)	252.0	52.0	200.0	370.0
	Low	16 (59.3%)	166.2	23.0	118.0	198.0
	High	0 (0.0%)	.	.	.	.
	Total	27 (100.0%)	201.2	56.5	118.0	370.0
Retinol binding protein (mg/L)	Normal	6 (25.0%)	31.7	12.5	10.0	49.0
	Low	18 (75.0%)	23.3	9.5	0.0	38.4
	High	0 (0.0%)	.	.	.	.
	Total	24 (100.0%)	25.4	10.7	0.0	49.0
Transferrin (g/L)	Normal	21 (91.3%)	2.5	0.2	2.2	3.0
	Low	1 (4.3%)	2.0	.		
	High	1 (4.3%)	3.9	.		
	Total	23 (100.0%)	2.6	0.4	2.0	3.9
C-reactive protein (nmol/L)	Normal	30 (96.8%)	4.2	3.0	0.3	11.4
	Low	0 (0.0%)	.	.	.	.
	High	1 (3.2%)	11.1	.		
	Total	31 (100.0%)	4.5	3.2	0.3	11.4

Table S51. Plasmatic proteins - classification according to reference values; mean, standard-deviation, minimum and maximum (units mentioned in table).

Performed analysis per type of MPS: total protein (four MPS I; three MPS II; nine MPS III; three MPS IV; eight MPS VI); albumin (all patients); ferritin (four MPS I; four MPS II; nine MPS III; three MPS IV; nine MPS VI; one MPS VII); pre-albumin (five MPS I; four MPS II; six MPS III; two MPS IV; nine MPS VI; one MPS VII); RBP (five MPS I; three MPS II; five MPS III; two MPS IV; eight MPS VI; one MPS VII); transferrin (five MPS I; two MPS II; five MPS III; one MPS IV; nine MPS VI; one MPS VII); C-reactive protein (five MPS I; four MPS II; nine MPS III; three MPS IV; nine MPS VI; one MPS VII)

Lipids		n (%)	Mean	SD	Min	Max
Total cholesterol	Normal	30 (96.8%)	151.1	28.0	109.0	222.0
	High	1 (3.2%)	208.0	.	208.0	208.0
	Total	31 (100.0%)	153.0	29.4	109.0	222.0
LDL – Cholesterol	Normal	28 (90.3%)	92.0	22.0	48.0	160.0
	High	3 (9.7%)	137.7	11.7	129.0	151.0
	Total	31 (100.0%)	96.4	25.2	48.0	160.0
HDL – Cholesterol	Normal	14 (45.2%)	47.0	11.4	24.0	70.0
	Low	15 (48.4%)	34.3	9.9	10.0	48.0
	High	2 (6.5%)	71.0	7.1	66.0	76.0
	Total	31 (100.0%)	42.4	14.2	10.0	76.0
Triglycerides	Normal	29 (93.5%)	76.5	33.1	39.0	147.0
	Low	1 (3.2%)	36.0	.	36.0	36.0
	High	1 (3.2%)	159.0	.	159.0	159.0
	Total	31 (100.0%)	77.8	36.1	36.0	159.0

Table S52. Lipid profile (mg/dL) - classification according to reference values and mean, standard-deviation, minimum and maximum (n=31).

Essential fatty acids (reference values)		n (%)	Mean	SD	Min	Max
Araquidonic acid	Normal	8 (66.7%)	194.3	37.5	136.0	240.0
C20:4w6	Low	0 (0.0%)	.	.	.	.
(105.0-244.0)	High	4 (33.3%)	299.8	46.5	270.0	369.0
	Total	12 (100.0%)	229.4	64.7	136.0	369.0
Docosahexaenoic acid C22:6w3	Normal	10 (83.3%)	45.2	17.6	26.8	77.8
(4.4-30.0)	Low	2 (16.7%)	18.4	4.4	15.3	21.5
	High	0 (0.0%)	.	.	.	.
	Total	12 (100.0%)	40.7	19.1	15.3	77.8
Eicosapentaenoic acid C20:5w3	Normal	12 (100.0%)	12.7	6.0	4.6	25.0
(25.9-84.2)	Low	0 (0.0%)	.	.	.	.
	High	0 (0.0%)	.	.	.	.
	Total	12 (100.0%)	12.7	6.0	4.6	25.0

Table S53. Plasma essential fatty acids levels, in µg/mL - classification according to reference values; mean, standard-deviation, minimum and maximum (n=12: three MPS I; four MPS III; two MPS IV; three MPS VI).

Vitamins		n (%)	Mean	SD	Min	Max
Folic acid	Normal	17 (85.0%)	22.8	10.0	11.0	41.0
(nmol/L)	Low	1(5.0%)	4.0	.	4.0	4.0
	High	2 (10.0%)	49.5	6.4	45.0	54.0
	Total	20 (100.0%)	24.5	13.3	4.0	54.0
Vitamin B_{12}	Normal	19 (63.3%)	440.9	134.8	691.0	216.0
(pmol/L)	Low	0(0.0%)	.	.	.	.
	High	11 (36.7%)	721.6	108.1	884.0	542.0
	Total	30 (100.0%)	543.8	185.0	884.0	216.0
Vitamin A	Normal	17 (73.9%)	1.3	0.4	0.8	2.1
(µmol/L)	Low	6 (26.1%)	0.7	0.2	0.4	1.0
	High	0 (0.0%)	.	.	.	.
	Total	23 (100.0%)	1.2	0.4	0.4	2.1
Vitamin E	Normal	13 (59.1%)	19.3	4.5	12.8	26.9
(µmol/L)	Low	6 (27.3%)	18.3	3.9	13.9	23.9
	High	3 (13.6%)	29.0	8.1	22.8	38.2
	Total	22 (100.0%)	20.3	5.9	12.8	38.2

Table S54. Plasma vitamins (units mentioned in table) - classification according to reference values; mean, standard-deviation, minimum and maximum.*

Performed analysis per type of MPS: folic acid (three MPS I; two MPS II; eight MPS III; one MPS IV; five MPS VI; one MPS VII); vitamin B_{12} (five MPS I; three MPS II; nine MPS III; three MPS IV; nine MPS VI; one MPS VII); Vitamin A (four MPS I; one MPS II; seven MPS III; three MPS IV; eight MPS VI); vitamin E (four MPS I; one MPS II; seven MPS III; three MPS IV; seven MPS VI).

(*) Vitamin D – in the phosphocalcium metabolism section

Minerals		n (%)	Mean	SD	Min	Max
Magnesium	Normal	21 (77.8%)	0.84	0.07	0.70	0.93
(mmol/L)	Low	4 (14.8%)	0.74	0.03	0.70	0.77
	High	2 (7.4%)	0.95	0.12	0.86	1.03
	Total	27 (100.0%)	0.83	0.08	0.70	1.03
Selenium	Normal	8 (72.7%)	0.87	0.35	0.18	1.30
(mmol/L)	Low	3 (27.3%)	0.35	0.22	0.10	0.53
	High	0 (0.0%)	.	.	.	.
	Total	11 (100.0%)	0.73	0.40	0.10	1.30
Zinc	Normal	23 (95.8%)	14.0	2.3	10.7	20.5
(µmol/L)	Low	1 (4.2%)	9.5	.		
	High	0 (0.0%)	.	.	.	.
	Total	24 (100.0%)	13.8	2.5	9.5	20.5
Potassium	Normal	24 (92.3%)	4.4	0.7	1.5	5.5
(mmol/L)	Low	0 (0.0%)	.	.	.	.
	High	2 (7.7%)	6.1	0.7	5.6	6.6
	Total	26 (100.0%)	4.5	0.9	1.5	6.6
Sodium	Normal	24 (85.7%)	138.8	2.0	135.0	142.0
(mmol/L)	Low	4 (14.3%)	133.0	2.8	129.0	135.0
	High	0 (0.0%)	.	.	.	.
	Total	28 (100.0%)	138.0	2.9	129.0	142.0

Table S55. Plasma minerals (units mentioned in table) - classification according to reference values; mean, standard-deviation, minimum and maximum.
Performed analysis per type of MPS: magnesium (four MPS I; three MPS II; eight MPS III; three MPS IV; eight MPS VI; one MPS VII); selenium (three MPS I; three MPS III; two MPS IV; three MPS VI); zinc (three MPS I; three MPS II; six MPS III; two MPS IV; eight MPS VI; one MPS VII); potassium (three MPS I; three MPS II; nine MPS III; three MPS IV; seven MPS VI; one MPS VII); sodium (four MPS I; three MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII).

Liver function		n (%)	Mean	SD	Min	Max
Alanine transaminase	Normal	23 (79.3%)	27.8	7.0	18.0	46.7
	Low	0 (0.0%)	.	.	.	.
	High	6 (20.7%)	52.9	17.3	34.7	79.0
	Total	29 (100.0%)	33.0	14.1	18.0	79.0
Aspartate transaminase	Normal	24 (77.4%)	20.6	8.6	10.8	47.9
	Low	0 (0.0%)	.	.	.	.
	High	7 (22.6%)	69.0	15.5	52.1	95.2
	Total	31 (100.0%)	31.5	23.0	10.8	95.2
Gamma-glutamyl transferase	Normal	18 (64.3%)	19.5	11.2	9.0	44.9
	Low	7 (25.0%)	9.8	1.7	6.0	10.8
	High	3 (10.7%)	375.5	499.7	70.1	952.1
	Total	28 (100.0%)	55.2	177.1	6.0	952.1

Table S56. Liver function (IU/L): classification according to reference values and mean, standard-deviation, minimum and maximum.
Performed analysis per type of MPS: alanine transaminase (five MPS I; four MPS II; nine MPS III; three MPS IV; nine MPS VI; one MPS VII); aspartate transaminase (four MPS I; four MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII); gamma-glutamyl transferase (four MPS I; three MPS II; nine MPS III; three MPS IV; eight MPS VI; one MPS VII).

Renal function		n (%)	Mean	SD	Min	Max
Urea ***plasma***	Normal	28 (90.3%)	5.0	2.4	1.5	12.1
(mmol/L)	Low	1 (3.2%)	1.1	.		
	High	2 (6.5%)	7.4	1.1	6.6	8.1
	Total	31 (100.0%)	5.1	2.4	1.1	12.1
Creatinine ***plasma***	Normal	7 (22.6%)	38.0	9.4	26.0	49.0
(µmol/L)	Low	24 (77.4%)	29.8	9.5	12.0	53.0
	High	0 (0.0%)	.	.	.	.
	Total	31 (100.0%)	31.7	9.9	12.0	53.0

Table S57. Renal function: classification according to reference values; mean, standard-deviation, minimum and maximum (units mentioned in table) (n=31).

Total blood count		n (%)	Mean	SD	Min	Max
Haemoglobin	Normal	26 (83.9%)	133.3	8.4	116.0	148.0
(g/L)	Low	5 (16.1%)	120.6	5.0	113.0	126.0
	High	0 (0.0%)	.	.	.	.
	Total	31 (100.0%)	131.2	9.2	113.0	148.0
Leucocytes	Normal	27 (87.1%)	6.3	1.3	4.6	10.7
($x10^3/\mu L$)	Low	2 (6.5%)	3.3	0.5	2.9	3.6
	High	2 (6.5%)	13.7	2.7	11.8	15.6
	Total	31 (100.0%)	6.6	2.4	2.9	15.6
Lymphocytes	Normal	21 (77.8%)	2.8	1.1	1.3	6.8
($x10^3/\mu L$)	Low	1 (3.7%)	3.1	.		
	High	5 (18.5%)	3.8	1.5	2.3	6.3
	Total	27 (100.0%)	3.0	1.2	1.3	6.8
Platelets	Normal	22 (71.0%)	224.8	59.1	84.0	347.0
($x10^3/\mu L$)	Low	7 (22.6%)	162.4	53.2	78.0	248.0
	High	2 (6.5%)	536.0	36.8	510.0	562.0
	Total	31 (100.0%)	230.8	101.9	78.0	562.0

Table S58. Total blood count: classification according to reference values; mean, standard-deviation, minimum and maximum (units mentioned in table).
Performed analysis per type of MPS: haemoglobin, leucocytes and platelets (all sample); lymphocytes (five MPS I; four MPS II; six MPS III; two MPS IV; nine MPS VI; one MPS VII).

Phosphocalcium metabolism		n (%)	Mean	SD	Min	Max
Calcium	Normal	25 (86.2%)	2.35	0.08	2.20	2.55
(mmol/L)	Low	2 (6.9%)	2.09	0.02	2.07	2.10
	High	2 (6.9%)	2.54	0.01	2.53	2.55
	Total	29 (100.0%)	2.34	0.12	2.07	2.55
Phosphorus	Normal	24 (82.8%)	1.45	0.21	0.94	1.87
(mmol/L)	Low	0 (0.0%)	.	.	.	.
	High	5 (17.2%)	1.57	0.08	1.49	1.68
	Total	29 (100.0%)	1.47	0.20	0.94	1.87
Vitamin D	Normal	4 (12.9%)	82.5	3.2	79.9	86.6
(nmol/L)	Insufficient	12 (38.7%)	63.0	7.9	52.4	74.9
	Deficient	15 (48.4%)	26.7	11.7	10.0	46.0
	Total	31 (100.0%)	47.9	23.7	10.0	86.6
Alkaline phosphatase	Normal	21 (77.8%)	2.22	1.14	0.30	4.40
(µkat/L)	Low	3 (11.1%)	2.07	0.50	1.60	2.60
	High	3 (11.1%)	2.37	0.12	2.30	2.50
	Total	27 (100.0%)	2.22	1.01	.30	4.40
Parathormone	Normal	19 (82.6%)	4.32	1.52	2.40	7.70
(pmol/L)	Low	0 (0.0%)	.	.	.	.
	High	4 (17.4%)	11.07	2.48	8.59	14.50
	Total	23 (100.0%)	5.50	3.09	2.40	14.50
Calcium/creatinine	Normal	17 (94.4%)	0.20	0.15	0.52	0.01
	Low	0 (0.0%)	.	.	.	.
	High	1 (5.6%)	0.89	.	0.89	0.89
	Total	18 (100.0%)	0.24	0.22	0.89	0.01
Tubular reabsorption of phosphate	Normal	17 (100.0%)	0.95	0.06	0.99	0.80
	Low	0 (0.0%)	.	.	.	.
	High	0 (0.0%)	.	.	.	.
	Total	17 (100.0%)	0.95	0.06	0.99	0.80

Table S59. Phosphocalcium metabolism analytical indicators. Classification according to reference values; mean, standard-deviation, minimum and maximum (units mentioned in table).
Performed analysis per type of MPS: calcium and phosphorus (five MPS I; four MPS II; eight MPS III; three MPS IV; nine MPS VI); alkaline phosphatase (four MPS I; four MPS II; seven MPS III; three MPS IV; nine MPS VI); parathormone (four MPS I; four MPS II; four MPS III; two MPS IV; nine MPS VI); calcium / creatinine (four MPS I; one MPS II; four MPS III; one MPS IV; eight MPS VI); tubular reabsorption of phosphate (four MPS I; one MPS II; four MPS III; eight MPS VI).

Items	N	Mean	SD	Median	Minimum	Maximum
1. Eat using fingers to pick up food item	31	3.2	4.6	0	0	11
2. Scoop with a spoon and bring it to mouth	30	4.3	4.9	1	0	11
3. Use a fork for large, easy to pierce foods	30	5.2	4.9	5	0	11
4. Use a knife to butter bread or cut soft foods	29	6.6	4.9	9	0	11
5. Drink from an open jar up held securely with one hand	30	5.2	5.4	3	0	11
6. Pour liquid from carton or jug	29	6.7	4.5	8	0	11
7. Open a jar or food container by twisting lid	29	8.7	3.3	11	0	11

Table S60. HAQ: activities related to eating and drinking (ranging from 0 "not difficult at all" to 11 points "unable to do").

Items	N	Mean	SD	Median	Minimum	Maximum
8. Put on a T-shirt, dress or jumper	29	7.5	4.4	10.0	0	11
9. Put on trousers with an elasticated waist	30	7.5	4.6	10.5	0	11
10. Put on a front-opening shirt, not including fasteners	30	7.3	4.8	11.0	0	11
11. Tuck shirt into back of trousers by reaching back with hand	29	8.3	4.2	11.0	0	11
12. Button and unbutton large buttons	30	8.3	4.1	11.0	0	11
13. Zip and unzip, including separating or hooking up the zip	28	7.6	4.5	11.0	0	11
14. Put on socks	30	8.8	3.8	11.0	0	11
15. Put on an unfastened or slip-on shoe	27	7.4	4.7	11.0	0	11
16. Tie shoelaces	30	9.1	3.7	11.0	0	11

Table S61. HAQ: activities related to dressing (ranging from 0 "not difficult at all" to 11 points "unable to do").

Items	N	Mean	SD	Median	Minimum	Maximum
17. Turn tap on and off	29	5.4	5.3	5.0	0	11
18. Wash and dry upper body thoroughly (neck, arms, chest, upper back)	29	8.3	4.2	11.0	0	11
19. Wash and dry lower body thoroughly (abdomen, lower back, legs, feet)	28	8.0	4.4	11.0	0	11
20. Brush or comb hair thoroughly (including hair on top of head and back of neck)	19	9.1	3.7	11.0	0	11
21. Cut fingernails with clippers	28	9.6	3.3	11.0	0	11
22. Prepare toothbrush with toothpaste	30	5.9	5.2	8.5	0	11
23. Brush teeth, even if not thoroughly	29	5.6	5.3	4.0	0	11

Table S62. HAQ: activities related to bathing (ranging from 0 "not difficult at all" to 11 points "unable to do").

Items	N	Mean	SD	Median	Minimum	Maximum
24. Manage clothes before and after toileting	29	6.8	4.9	11.0	0	11
25. Manage toilet seat, get toilet paper and flush toilet	29	6.6	5.1	11.0	0	11
26. Get on and off toilet without assistance	29	5.3	5.3	5.0	0	11
27. Wipe self thoroughly after bowel movements	28	7.4	4.7	11.0	0	11

Table S63. HAQ: activities related to toileting (ranging from 0 "not difficult at all" to 11 points "unable to do").

Items	N	Mean	SD	Median	Minimum	Maximum
28. Retrieve objects from floor while sitting	30	6.5	4.6	8.0	0	11
29. Get on and off the floor	31	6.1	5.0	10.0	0	11
30. Manage a seat belt or restraint in car	29	6.7	4.8	11.0	0	11
31. Get in and out of the front seat of a car	30	5.9	4.9	5.5	0	11
32. Open and close a car door without assistance	30	6.2	5.1	8.0	0	11

Table S64. HAQ: activities related to mobility (ranging from 0 "not difficult at all" to 11 points "unable to do").

Items	N	Mean	SD	Median	Minimum	Maximum
35. Walk length of shopping centre or through supermarket aisles	30	5.9	4.9	8.0	0	11
36. Walk across level surfaces, such as smooth pavements or driveways	30	5.7	4.8	6.5	0	11
37. Walk across rough or uneven surfaces, such as a lawn or a gravel driveway	30	6.7	4.8	10.0	0	11
38. Step on and off curbs	30	6.6	4.7	9.5	0	11
39. Walk up a full flight of stairs (12-15 steps) holding a rail and/or stopping between steps	30	6.3	4.8	8.0	0	11

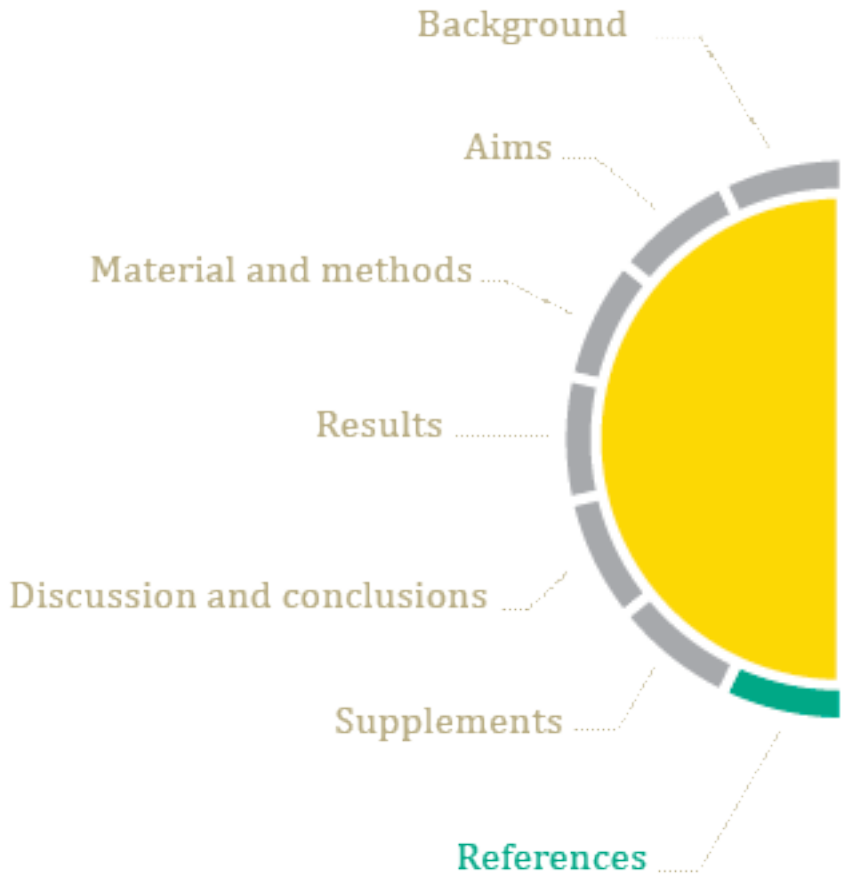
Table S65. HAQ: activities related to walking and climbing stairs (ranging from 0 "not difficult at all" to 11 points "unable to do").

Domain	N	Mean	SD	Median	Minimum	Maximum
Eating and drinking	31	5.5	4.0	4.4	0	11
Dressing	29	8.0	3.9	10.4	0	11
Bathing	30	7,1	3,9	8,0	0	11
Toileting	29	6.5	4.8	9.5	0	11
Mobility	31	6.3	4.1	5.8	0	11
Walking and climbing stairs	30	6,3	4,5	7,0	0	11
All domains	31	6.6	3.8	6.8	0	11

Table S66. HAQ: all domains (ranging from 0 "not difficult at all" to 11 points "unable to do").

Activity domain	Independent		Minimal assistance		Moderate assistance		Complete assistance		Median
	n	(%)	n	(%)	n	(%)	n	(%)	Mdn
Eating	3	(10.3%)	4	(13.8%)	5	(17.2%)	**17**	**(58.6%)**	4
Grooming	4	(13.8%)	3	(10.3%)	6	(20.7%)	**16**	**(55.2%)**	4
Bathing	2	(6.9%)	2	(6.9%)	6	(20.7%)	**19**	**(65.5%)**	4
Dressing upper body	5	(17.2%)	2	(6.9%)	4	(13.8%)	**18**	**(62.1%)**	4
Dressing lower body	4	(13.8%)	4	(13.8%)	2	(6.9%)	**19**	**(65.5%)**	4
Toileting	8	(27.6%)	3	(10.3%)	3	(10.3%)	**15**	**(51.7%)**	4
Chair and toilet transfers	**12**	**(41.4%)**	2	(6.9%)	4	(13.8%)	11	(37.9%)	3
Car transfers	8	(27.6%)	3	(10.3%)	4	(13.8%)	**14**	**(48.3%)**	3
Bed mobility / transfers	**13**	**(44.8%)**	2	(6.9%)	3	(10.3%)	11	(37.9%)	2
Tub transfers	6	(20.7%)	3	(10.3%)	6	(20.7%)	**14**	**(48.3%)**	3
Indoor locomotion	9	(31.0%)	4	(13.8%)	3	(10.3%)	**13**	**(44.8%)**	3
Outdoor locomotion	7	(25.0%)	4	(14.3%)	2	(7.1%)	**15**	**(53.6%)**	4
Stairs	10	(34.5%)	1	(3.4%)	4	(13.8%)	**14**	**(48.3%)**	3

Table S67. HAQ: relative and absolute frequency of answers about autonomy (caregiver assistance), by domain. Median is indicated and mode is evidenced in bold.


Background
Aims
Material and methods
Results
Discussion and conclusions
Supplements
References

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